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# *The Journal* *of* *Laboratory and Clinical* *Medicine*

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# *The Journal of Laboratory and Clinical Medicine*

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## *ORIGINAL ARTICLES*

### AN EXPERIMENTAL INVESTIGATION OF CERTAIN FEATURES OF THE PHARMACOLOGICAL ACTION OF SALVARSAN\*

BY D. E. JACKSON, PH.D., M.D., AND G. RAAP, A.B., A.M., CINCINNATI, OHIO

IN a series of experiments performed at the Hygienic Laboratory in Washington in the year 1918 it was shown by Jackson and Smith<sup>1</sup> that one of the most important and outstanding features of the acute symptoms of poisoning following the intravenous injection of arsphenamine solutions in dogs consists in the production of a very marked and prolonged rise in the pulmonary blood pressure. This within itself would perhaps be sufficient to account for a part, if not for all, of the milder toxic symptoms which are occasionally produced clinically by the injection of arsphenamine. But aside from the pulmonary vascular changes, there remained the possibility that the dyspnea and marked respiratory disturbances which are frequently present during "nitritoid crises" of severe, acute arsphenamine intoxication might be due to, or associated with, a marked bronchial constriction. This point was not investigated by Jackson and Smith although at that time the presence of some such factor as this was strongly suspected, particularly on account of the analogy in action on the bronchioles which is often exhibited among metallic salts. In the present work we have carried out some preliminary experiments in order to determine whether or not any true bronchial asthmatic action is produced by injections of arsphenamine.

The solutions used by us have been made up from "salvarsan" as produced by the H. A. Metz Laboratories in New York. Mr. Metz has very kindly supplied us with a quantity of "salvarsan" of lot No. H56. This was a particularly good batch as had been previously shown by laboratory tests and by extensive clinical use. Generally our solutions have been made up to 2 per cent strength of salvarsan, and the amount of alkali used in neutralizing the dihydrochloride

\*From the Department of Pharmacology of the University of Cincinnati Medical School, Cincinnati, Ohio.

salt has been sufficient to produce the disodium salt, and in most instances a further slight excess of alkali has been added. In a few cases we used mixtures of the mono- and di-sodium salts. Fresh solutions were always made up only a few minutes before they were injected into the animal.

Figs. 1, 2, and 3 show at once the action which salvarsan has on the systemic blood pressure (lower tracing) and on the bronchial musculature. The lung

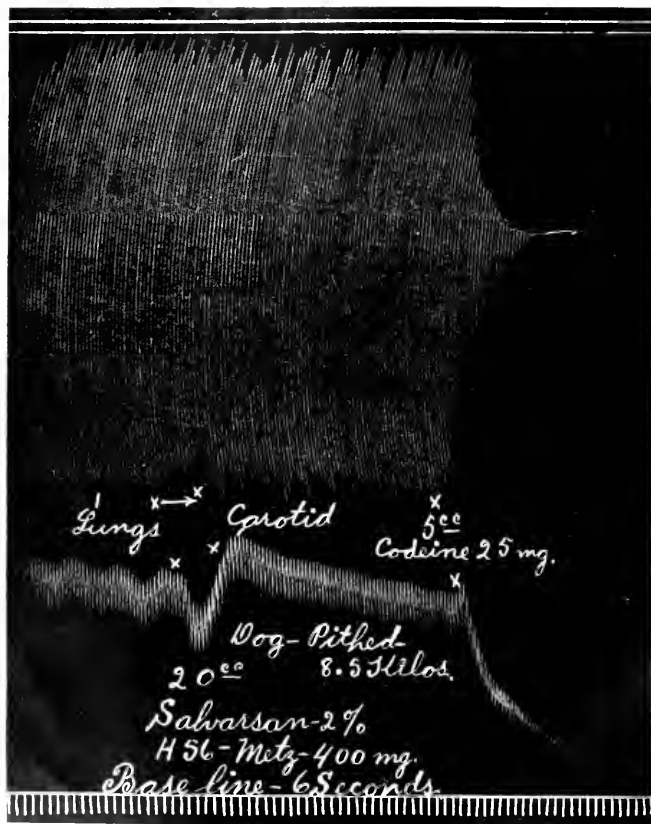


Fig. 1.

tracings in the experiments were made by means of a special method<sup>2</sup> in which the air was intermittently aspirated from the chest cavity while the tracing was made on a tambour connected with the side tube of the tracheal cannula. The dogs were pithed in each case. In tracing 1, it is seen that 20 c.c. of 2 per cent solution injected into a dog weighing 8.5 kilos produced practically no

effect at all on the bronchioles, either in the nature of contraction or dilatation. Fig. 2 shows a moderate contraction of the bronchioles as indicated by the slight reduction in amplitude of the respiratory tracing. (It should be noted here that the pulmonary pressure of this animal undoubtedly rose to a great height following the injection of the salvarsan.) Near the end of this tracing an injection

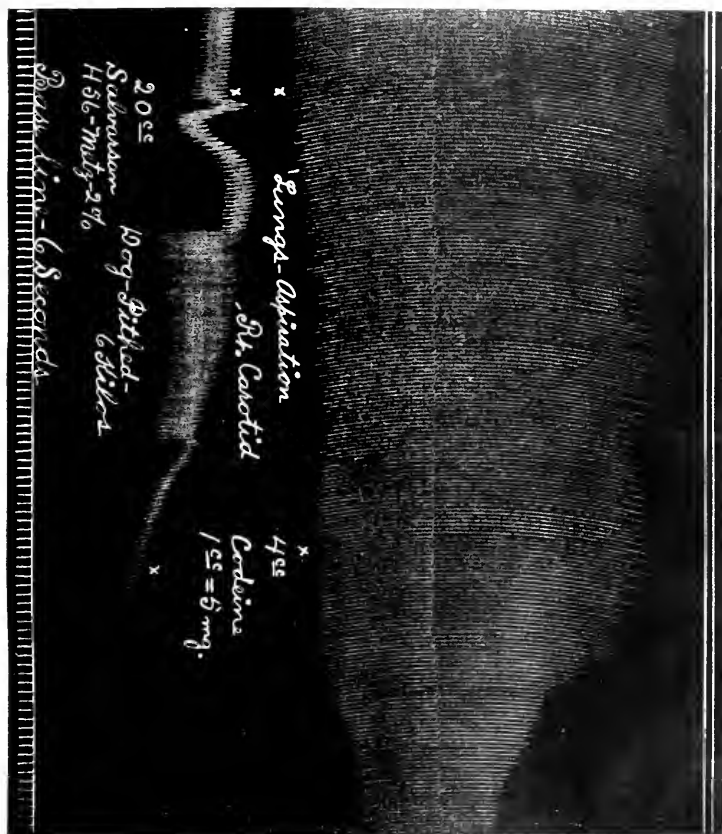


Fig. 2.

of 4 c.c. of codeine sulphate (20 milligrams) was made. This produced a marked contraction of the bronchioles and was intended to be a check on the technic of the experiment in order to show that the apparatus, the lungs, etc., were all working properly. Fig. 3 is a similar experiment in which 20 c.c. of salvarsan caused a slight dilatation of the bronchioles. These experiments show that good

preparations of salvarsan do not cause a marked contraction of the bronchioles. But, on the other hand, they do not show that especially toxic preparations might not produce very serious results in this direction. Obviously this point should be investigated further, and with a much larger range of samples of arsphenamine than we have had at our command in the present investigations. A num-

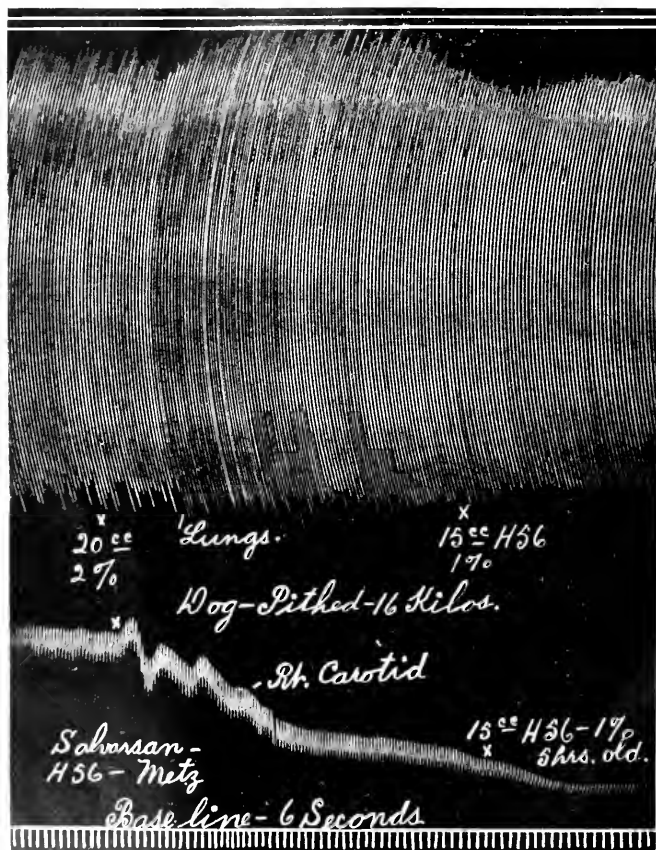
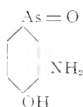


Fig.

ber of other acetarsphenamine compounds produced in the manufacture of arsphenamine were examined by Jackson and Smith, but it appeared that none of those examined at 1:10000 could be responsible for severe, acute symptoms following intravenous injections. But in a later paper by Smith<sup>2</sup> it was shown that one of the acetarsphenamine compounds, namely amino hydroxy-phenyl-arsenoxide,





which is an oxidation product of arsphenamine, affected the pulmonary blood pressure in a manner quite comparable with that of a solution of arsphenamine of corresponding strength. "The arsenoxide content of arsphenamine varies usually between 0.5 and 2 per cent. Occasionally a preparation is encountered

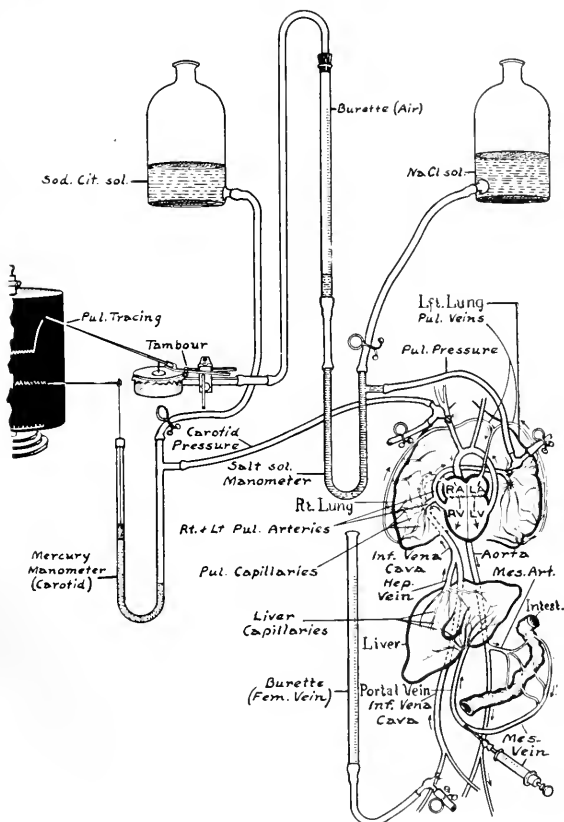


Fig. 4.

that contains as high as 5 per cent arsenoxide (Dr. C. N. Myers, quoted by Smith) and such a preparation might very readily be highly toxic owing solely to its arsenoxide content." In a recent article by Schamberg, Kolmer and Raiziss<sup>4</sup> the presence in some arsphenamine and neoarsphenamine preparations

of an unidentified toxic substance designated by them as "X" has been emphasized. And Stokes and Busman<sup>1</sup> have reported toxic reactions following injections of arsphenamine through a certain brand of so-called pure gum rubber tubing when this is new, but not after the tubing has been used for a short while. It is obvious that such factors as these might possibly cause a severe, or even fatal, bronchoconstriction in very susceptible patients, when any such constrict-

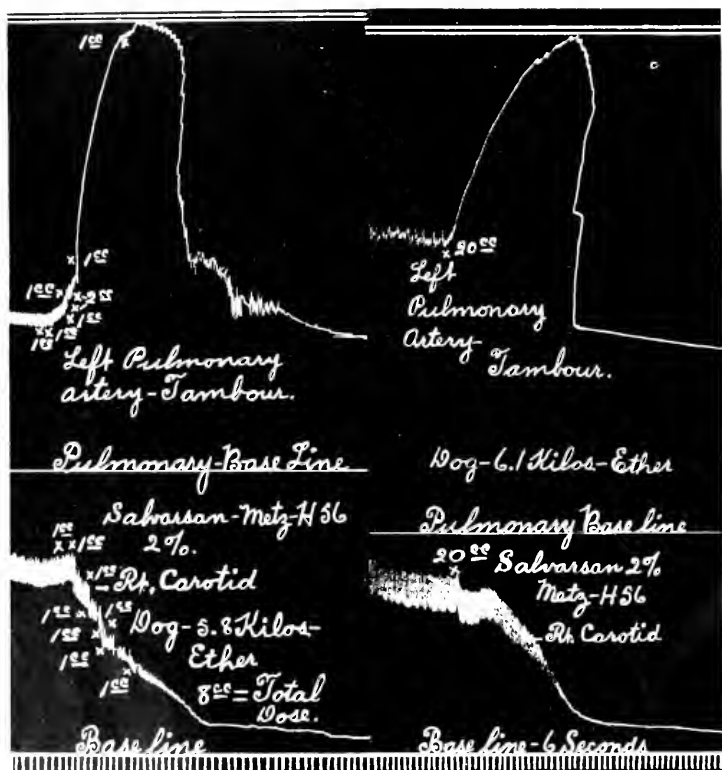


Fig. 3

tion was complicated by the simultaneous presence of a great rise in the pulmonary arterial pressure. Unfortunately it will require many more experiments before all such obscuring phenomena as these can be fully investigated. But the present experiments have been sufficient to show that any dangerous bronchoconstriction is not to be feared with the proper use of first class preparations of arsphenamine. (See also Hanzlik and Karsner<sup>4</sup>.)

Bearing in mind the evident rise in pulmonary arterial pressure after arsphenamine injections, as first demonstrated by Jackson and Smith,<sup>1</sup> and which was further investigated by Smith<sup>2</sup> alone, we have attempted in the present work to investigate further certain features of this important reaction. We have accordingly devised a very sensitive method for detecting very minute changes



Fig.

in the pulmonary pressure. The arrangement of the apparatus as used by us is diagrammatically illustrated in Fig. 4. In this illustration it will be seen that a cannula tied into the left pulmonary artery was connected with a manometer by rubber tubing. The chest was opened widely and artificial respiration was maintained throughout the experiment. Ether was administered by means of a special sight-feed device<sup>3</sup> which has been described by us elsewhere. The manom-

eter and the rubber tubing connecting with the pulmonary artery were all filled with normal salt solution (0.8 per cent sodium chloride in water). This solution has worked very well for us, and clotting has been very much less troublesome than was the case when we used sodium citrate solution. This latter is very poisonous and easily stops the heart if a small amount gets back into the right ventricle. Sodium chloride solution does not affect the heart. The top of the distal limb of the manometer was connected by rubber tubing to a burette of 50 c.c. capacity. The salt solution reached only a little way up in the burette, the upper part of which contained air and was connected by means of glass and rubber tubing to a tambour having a bowl about two inches in diameter. The tambour was very sensitive and thus readily recorded on the drum very minute changes in the pulmonary pressure. Carotid pressure was recorded in the usual manner with a mercury manometer.

Fig. 5 shows the results in two different dogs of injections of salvarsan solution, as recorded from the pulmonary (upper) and carotid (lower) arteries. It will be seen that the pulmonary pressure rose abruptly to a great height and that it did not fall until the carotid pressure reached a very low level. In the left hand tracing only 8 c.c. in all was injected into a small dog, yet this killed the animal. In the right hand tracing 20 c.c. of solution was fatal.

Fig. 6 shows a profound and lasting rise in pulmonary pressure following injection of 20 c.c. of 2 per cent salvarsan solution. It will be noted here that the carotid pressure remained at almost the normal height for a considerable time after the injection of the drug, which was carried out rapidly. And again the pulmonary pressure remained very high until the heart had reached an extremely weakened condition.

From Figs. 5 and 6 it will be seen that sudden intravenous injections of salvarsan produce their chief circulatory results primarily in the lungs. From a clinical standpoint it is interesting to speculate as to what symptoms such an action as this might produce in the patient. And Fig. 6 shows further that an ordinary blood pressure determination as recorded from the arm might prove very deceptive so far as showing the real condition of the entire circulatory system was concerned. For here the general systolic pressure had *fallen* only a few millimeters at a time when the pulmonary pressure had *risen* to an enormous height. It will be noted, of course, that the dose and rate of injection here considerably exceeded that applying clinically. We have accordingly attempted to get some comparative insight into the matter by giving very small, consecutive injections as shown in Fig. 7. In this case 1 c.c. was injected and then, after an interval, a further 1 c.c., etc., was given. In this manner we are able to observe the immediate results following each separate small dose. It is seen that 1 c.c. causes a very considerable rise in pulmonary pressure. The second 1 c.c. dose still further increases this rise, as does each of the succeeding injections. And it will be seen that the systemic pressure actually rose following the first injection. Five injections of 1 c.c. each and one injection of 3 c.c. (8 c.c. in all) finally brought the pulmonary pressure almost to the limit of its capacity to rise. And this process represented a duration of some minutes.

Figs. 5, 6, and 7 all well illustrate a peculiar phenomenon which appears to be always present in experiments involving the rapid injection of salvarsan so-

lutions. It will be noted that in each of these tracings the pulmonary tambour at the very beginning of the record exhibited marked excursions up and down. These excursions resulted from the respiratory movements of the lungs. The corresponding excursions can also be seen in the mercury manometer tracing from the carotid pressure. The speed of the drum was too slow here to show the blood pressure movements following each individual beat of the heart. But immediately after the injection of the drug the pulmonary pressure started to rise. At the same time the amplitude of the respiratory excursions of the tambour began to decrease, and as soon as a very high altitude was reached by the

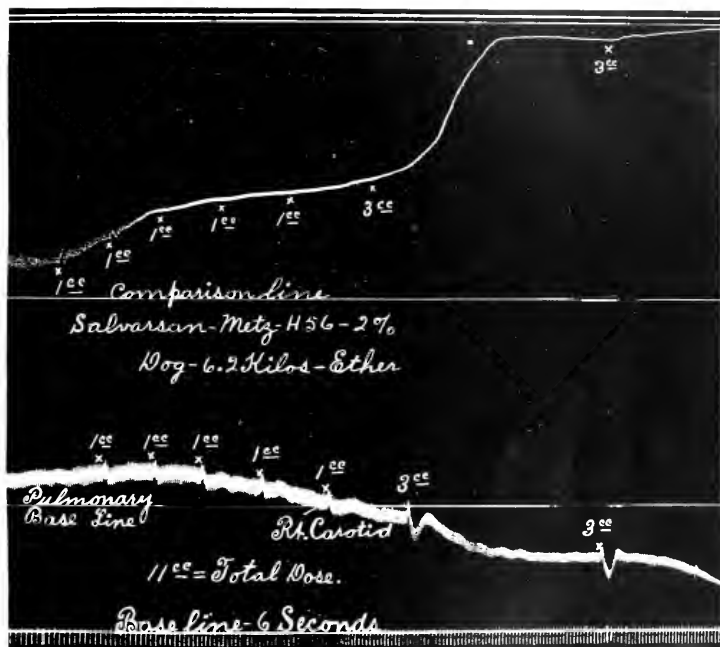


Fig. 7.

pressure the respiratory excursions were reduced to a minimum or disappeared altogether. But during all this period the respiratory inflation and deflation of the lungs remained constant, for this was carried out by means of an artificial respiration machine. Now let us ask, What is the cause of this peculiar change in the pulmonary blood pressure as reflected from the respiratory excursions of the lungs? For we have noted above that but little change was produced in the bronchial musculature by the salvarsan.

Fig. 8 probably illustrates a point having a bearing on this subject. In this tracing it is seen that three small injections of 2 c.c. each, produced a marked rise

in the pulmonary pressure but had only a slight effect on the carotid pressure. Following these, however, an injection of  $\frac{1}{2}$  c.c. of adrenaline (1-10,000) was given and this raised the carotid pressure but markedly lowered the pulmonary pressure. At the same time there was a slight tendency for the amplitude of the respiratory movements of the pulmonary tambour to increase, that is, to return toward the normal again. But as the effects of the adrenaline wore off the respiratory excursions of the tambour again became reduced. This same point is again illustrated, perhaps more markedly, in Fig. 9. This peculiar and un-

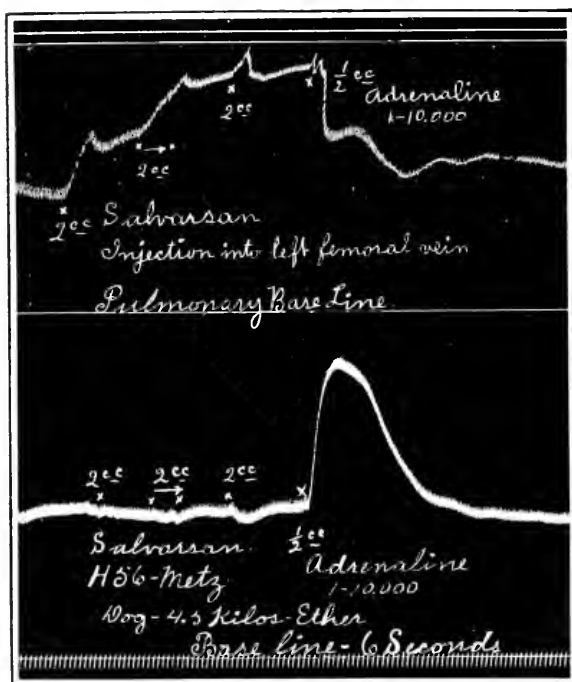


Fig. 8.

expected action of adrenaline calls to mind at once the various clinical recommendations which have been made by Milian,<sup>7</sup> Beeson,<sup>8</sup> and others regarding the use of adrenaline in cases of severe arsphenamine poisoning. And the relation which adrenaline bears to the spasmodic contraction of the bronchioles in acute anaphylaxis also reminds one of the various anaphylactic hypotheses by which different writers have attempted to explain the cause of arsphenamine poisoning. We have not been able to prove, however, that the phenomena which we have noted here as being produced by adrenaline in cases of experimental

acute salvarsan poisoning bear any direct relation to the clinical results which have been described as being produced by adrenaline injections in some cases of arsphenamine poisoning. On the other hand, the apparent improvement and lowering of the pulmonary pressure would undoubtedly be of benefit in these cases. We strongly suspect that the lowering of the pulmonary pressure here was due to a mechanical shifting of the blood from the venous to the arterial side of the circulatory system. This would result from contraction of the arte-

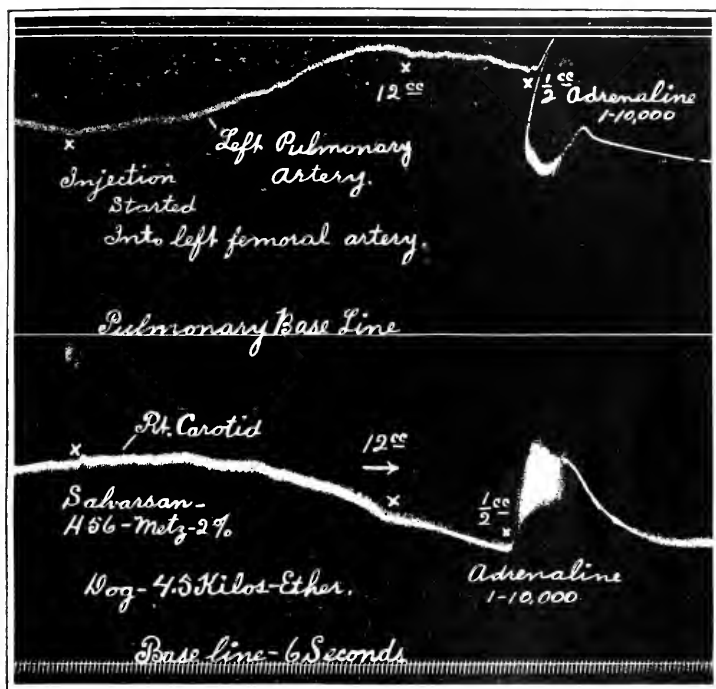


Fig. 9.

rioles. The direct action of adrenaline on the heart would also tend to improve the general character of the circulation. It would appear that when the pulmonary pressure is very high then the pulmonary arterioles, etc., are put on such a high tension that the regular respiratory movements of the lungs are not sufficient to cause much change in the relative movement and volume of blood in the pulmonary vessels, as indicated in the tracing made by the pulmonary tambour. Adrenaline causes a general shifting of the blood volume and thus indirectly affects the pulmonary pressure.

We wish now to take up another phase of the subject. It was long ago shown by Joseph<sup>9</sup> that acid solutions of arsphenamine could produce precipitation in the blood if the concentration of the drug exceeded 0.1 per cent. And Danysz<sup>10</sup> has attempted to show that precipitation of the arsphenamine occurs both *in vitro* and *in vivo* even with alkaline solutions. Smith<sup>3</sup> in the light of these, and other previous observations, has carefully studied this point with reference to the action of solutions of arsphenamine on serum *in vitro*. He finds that acid solutions (dihydrochloride) of arsphenamine produce very bulky precipitates in

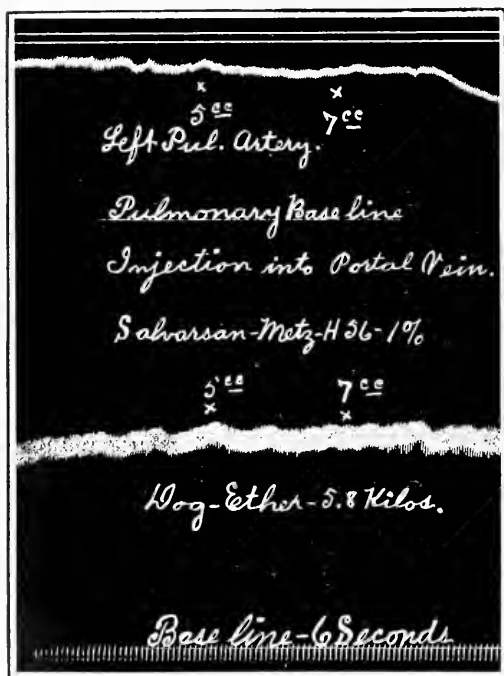


Fig. 10.

serum *in vitro*, and also cause a great rise in pulmonary pressure if injected intravenously. *In vitro* the precipitate between serum and the monosodium salt of arsphenamine varies from a distinct turbidity to a moderately heavy precipitate. But Smith found that no precipitate occurred *in vitro* between dog serum and alkaline arsphenamine solutions containing 0.8 c.c. or more normal sodium hydrate per 100 mg. of drug. Smith has also shown further that perfusion of the lungs with a solution of arsphenamine dihydrochloride in physiological salt solution causes a contraction of the pulmonary vessels and a consequent decrease in



the rate of outflow of the perfusion fluid. Since this occurs with acid arsphenamine solutions, it seems evident that the drug itself acts directly on the pulmonary arterioles to cause contraction, and that this is not entirely dependent on the alkali of the solutions as ordinarily used. Apparently then pulmonary vascular obstruction may be due to an extensive precipitate of the drug, to a specification of the drug itself on the muscle fibers of the arteriole walls, and to the presence of alkali in the solution used. In order to throw some further light on this question we have made injections of the drug into the femoral artery as shown in Fig. 9. In this case it will be seen that a dose of 12 c.c. produced a considerable rise in the pulmonary pressure. (The rise was really about twice

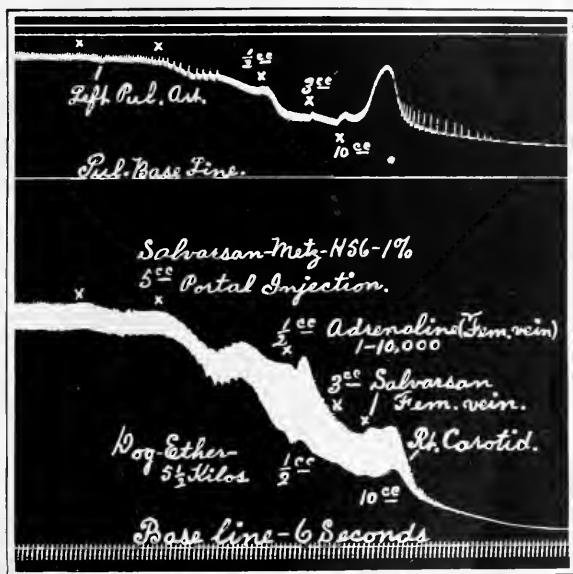


Fig. 11.

as great as the curve shows, for a slight leak in the metal bowl of the tambour was allowing air to escape very slowly throughout the tracing. This was discovered after the experiment was over.) We believe that in this case the drug (injected into the peripheral end of the femoral artery) simply washed out the blood from the artery and then passed directly on into the femoral vein without being precipitated out to any marked extent in the leg capillaries. This then, was almost equivalent to slow injection into the femoral vein directly.

We next proceeded to inject the salvarsan solution into a branch of the portal vein. In this case the solution had to pass through the liver capillaries as shown diagrammatically in Fig. 4. Fig. 10 shows the result of two such injec-

tions (of 5 c.c. and 7 c.c.). It will be noted that no rise occurred in the pulmonary pressure, but on the contrary some slight fall may have been produced.

Fig. 11 shows first an injection of 5 c.c. of salvarsan solution into the portal vein of a small dog. This produced an obvious fall in both the pulmonary and the carotid pressures. Following this  $\frac{1}{2}$  c.c. of adrenaline solution was given. This caused a slight fall of pulmonary pressure, but only a faint rise of the systemic pressure. As a check on the action of the apparatus, etc., two other injections (3 c.c. and 10 c.c.) of salvarsan solution were finally given by way of the femoral vein. The latter of these produced a very obvious rise in the pulmonary pressure. These experiments evidently show that the liver has removed

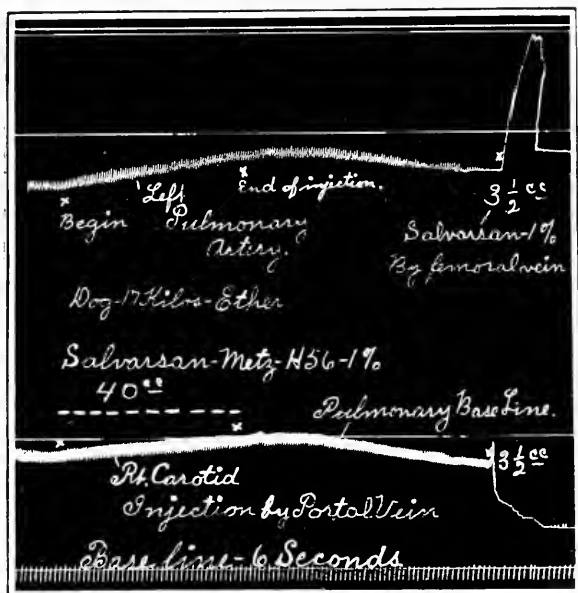


Fig. 12.

from the salvarsan solution its power to cause a rise in the pulmonary pressure. But on the contrary some portion of the drug must pass through the liver and on into the general circulation, for Fig. 11 shows that the salvarsan injections caused the systemic pressure to fall to zero and thus caused the death of the animal. This same point is further illustrated in Fig. 12. Here 40 c.c. of 1 per cent salvarsan solution was injected (between the points marked x, x). This dose by femoral vein would certainly have raised the pulmonary pressure to a great height. Here, however, only a gentle rise in the pulmonary pressure was produced, and this appears as if it might have been due simply to the addition of solution to the blood volume of the animal. But nevertheless, this dose of

the drug still exercised a very obvious toxic action on the animal. Near the end of the tracing an attempt was made to inject salvarsan into the femoral vein. Three and one-half c.c. were injected which started to produce an immediate rise in the pulmonary pressure, but unfortunately some air passed into the vein through the injecting cannula and the animal died of air embolism (verified at autopsy). The marked rise in the pulmonary tambour here, however, serves as a valuable check on the technic of the experiment and shows that any rise which the salvarsan might have produced in the pulmonary pressure would have been promptly recorded. We feel obliged to conclude, therefore, that if salvarsan solutions be injected into the portal vein, the passage of the drug through the liver will almost, if not totally, remove its power to raise the pulmonary pressure. It is probable that this action occurs to some extent, and this may be rather marked in some instances, in the case of the arterioles and capillaries of the leg also. But the liver seems to be much more effective in this direction than are the tissues of the leg.

It seems probable to us that this action of the liver results mainly, if not entirely, from a precipitation of the major portion of the salvarsan within the organ itself, perhaps in the form of emboli in the liver capillaries. The well-known detoxicating action which the liver manifests toward many poisons is not probably extensively concerned in this matter, at least not in the manner in which such detoxication is usually considered. There is a rather striking significance in the rapidity with which this precipitation must occur in the liver, if this is the correct explanation, for evidently only a very small proportion of the pulmonary pressure raising substance passes the liver, while at the same time very obvious effects from the drug may be produced in the carotid pressure immediately. This point perhaps has a bearing on the marked symptoms of liver disturbance, jaundice, etc., which are frequently manifested clinically in arsphenamine poisoning.

#### CONCLUSIONS

1. First-class preparations of salvarsan have almost no direct action on the bronchial musculature of the dog. It seems obvious that acute symptoms resembling anaphylactic shock, or the so-called "nitritoid crises," if produced by *good* preparations of salvarsan cannot be due to a spasmodic contraction of the bronchioles. But we are not sure that this action might not occur in the case of especially toxic samples of the drug.

2. We have studied the action of salvarsan on the pulmonary pressure by means of an especially sensitive method. We believe that even the smallest injections of salvarsan exercise some immediate action on the pulmonary pressure. Its detection depends only on the sensitivity of the method employed for its investigation.

3. When the pulmonary pressure has been greatly raised by salvarsan we have noted that injections of adrenaline tended to lower this pressure, and also to restore the excursions of the pulmonary pressure due to the respiratory movements of the lungs, when these had been previously greatly reduced by the salvarsan. We believe this results mainly from a mechanical shifting of the blood from the action of the adrenaline on the systemic vasculature.

4. When solutions of salvarsan are injected into the general circulation by way of the femoral artery the pulmonary blood pressure is still raised by the drug. But the rise in pressure is less than if the drug were injected by the femoral vein.

5. When solutions of salvarsan are injected into the portal vein and are thus carried through the liver before passing into the general circulation, then it is found that the drug produces but little if any effect on pulmonary pressure, although if the dosage is very large the pulmonary pressure may be raised slightly, apparently only as the result of an increased volume of fluid in the vessels. But toxic doses thus injected tend to lower the pulmonary pressure.

6. We believe this action of the liver is brought about by a precipitation of the drug in the capillaries and arterioles of the liver. This apparently does not correspond to the ordinary detoxicating action of the liver as manifested on many poisons.

7. This precipitation in the liver takes place quickly and it does not prevent some portion of the drug from passing on into the general circulation. For the systemic pressure may fall to a proportionately much greater degree than does the pulmonary pressure.

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## CHEMICAL CHANGES IN THE BLOOD IN DISEASE\*

### VII. CHLORIDES

BY VICTOR C. MYERS, PH.D., NEW YORK CITY

ALTHOUGH accurate data on the chloride content of the blood have been available longer than for the other blood constituents described in earlier papers of this series, its determination has not become a very common clinical procedure. This can hardly be ascribed to difficulties in the estimation of the chlorides, since it has been one of the simpler blood determinations, but must be attributed to either a lack of practical value in the test or to a nonappreciation of its importance, or possibly to both these factors.

Some of the observations recorded in the literature give the chloride content of the whole blood, others the content of the plasma or serum. Normally the chloride content of whole blood as sodium chloride amounts in round numbers to 0.45 to 0.50 per cent, while for the plasma the figures are about 0.12 per cent higher, i. e., 0.57 to 0.62 per cent. Since the plasma, rather than the whole blood, bathes the tissues of the body, it would seem more logical to study the chloride content of the plasma. Unfortunately, unless the plasma is quickly separated from the corpuscles there appears to be a gradual change (increase) in its chloride content owing to a passage of carbon dioxide from the plasma into the corpuscles (or its escape into the air) and of chlorides from the corpuscles to the plasma. In his paper dealing with the chlorides of blood plasma McLean<sup>1</sup> considered the influence of the plasma being allowed to stand in contact with the cells, but concluded that any change took place very slowly and that it was necessary only to centrifuge within two to three hours to avoid this danger. In their first paper on the plasma bicarbonate Van Slyke and Cullen<sup>2</sup> called attention to the effect which carbonic acid changes in whole blood might have on the chloride content of the plasma, a loss in bicarbonate resulting in an increase in the plasma chloride. This observation has recently been confirmed and extended by Fridericia,<sup>3</sup> who states that estimation of the chlorides of the plasma or serum from blood which has been kept in open receivers must give too low results, because chlorides have passed into the plasma (or serum) on account of the decreasing CO<sub>2</sub> tension. Similar observations regarding the increase in the plasma chlorides on standing have been made by Myers and Short.<sup>4</sup> This being the case, results obtained on whole blood would appear to be more trustworthy than those obtained on plasma. In any case a significant change in the blood chlorides should definitely affect the chloride content of whole blood as well as that of the plasma.

As far back as 1850 Carl Schmidt,<sup>5</sup> in his classic studies on the blood with special reference to cholera, gave figures for the chloride content of whole blood

\*From the Laboratory of Pathological Chemistry, New York Post-Graduate Medical School and Hospital, New York City.

TABLE I

OBSERVATIONS OF SCHMIDT ON THE CHLORIDE CONTENT OF WHOLE BLOOD AND PLASMA  
Recalculated as NaCl

CASE	AGE	SEX	CHLORIDES AS NaCl	
			WHOLE BLOOD per cent	PLASMA per cent
1. Normal	25	♂	0.437	0.589
2. "	20	♀	0.474	0.608
3. Cholera	26	♀	0.352	....
4. "	55	♂	0.431	....
5. "	20	♀	0.370	0.506
6. "	71	♂	0.367	0.492
7. "	23	♂	0.398	0.558
Diabetes	34	♂	0.461	0.572
4. Chronic edema with albuminuria	39	♂	0.552	0.646
5. Anasarca without albuminuria	42	♂	0.503	0.633

and plasma. Some of the observations recorded by Schmidt at that time have been recalculated to terms of sodium chloride and are given in Table I. The figures obtained in cholera appear to be low, while in the case of chronic edema with albuminuria there is a definite increase for both whole blood and plasma.

The French school was the first to emphasize the importance of the retention of chlorides in nephritis, particularly in those cases with edema, and to systematically employ restrictions of the chlorides in the diet in the treatment of these cases. The contributions of Widal and Javal<sup>6, 7</sup> are well known in this connection. These studies were later extended by Ambard,<sup>8, 9, 10</sup> who considered the relation of the chlorides of the serum to this general question, and constructed a formula<sup>11</sup> to express the rate of chloride excretion similar to his well-known formula for urea.

In this country McLean<sup>1</sup> has devoted considerable attention to this subject, working along lines somewhat similar to those of Ambard. In a fairly large series of normal individuals he found the plasma chloride to vary from 0.57 per cent to 0.62 per cent with a very constant chloride threshold of about 0.562 per cent. The threshold was calculated from the formula of Ambard and Weill<sup>11</sup> and confirms their observation on this point. McLean considered the question of the plasma chlorides in a number of pathologic conditions, the lowest observation being 0.50 per cent in a diabetic and the highest 0.84 per cent in a cardionephritic shortly before death. In general, relatively increased concentrations of chlorides were found in the plasma in certain forms of cardiac and renal disease, while decreased concentrations were noted in certain diabetic and fever patients, also after the action of digitalis, the decreased concentrations apparently resulting from a temporary or permanent lowering of the chloride threshold. Failure to excrete chlorides in pneumonia was found to be associated with a lowered concentration of chlorides in the plasma, excretion reappearing with a rise in the plasma chlorides. Edema was usually found to be accompanied by a relatively increased concentration of chlorides in the plasma, which ordinarily returned to the normal state with the disappearance of the edema.

During the present year Allen<sup>12</sup> has published a paper on arterial hypertension which has aroused considerable interest. He has endeavored to show that

salt retention (as shown by the plasma chloride) is the cause of pure hypertension, and also of the hypertension in many cases of kidney disease. This is a very fascinating explanation of hypertension, especially because restriction in the chloride intake affords such a simple means of treatment. Very recently Mosenthal and Short<sup>13</sup> have carried out in this hospital studies on cases of so-called pure hypertension with special reference to the relation of the blood chlorides to the blood pressure. So far these experiments have not given support to Allen's theory.

Höst<sup>14</sup> in a recent issue of this journal has presented a discussion of chloride metabolism with some interesting observations on several cases of acute nephritis. These cases showed edema, increased blood pressure, and a definite increase in the chloride concentration of the whole blood, figures ranging from 0.51 to 0.54 per cent. With improvement there was an increased chloride excretion and the blood chlorides returned to a normal concentration of 0.45 to 0.47 per cent.

A few illustrative findings for the chloride content of whole blood in nephritis, anemia, essential hypertension and diabetes are given in Table II. These are recent observations made with the method described below. (I am indebted to Dr. Killian for many of the figures.) In general it will be noted that the chlorides were high in most of the cases of nephritis, but were practically normal in the cases of essential hypertension and diabetes, although with the last mentioned the figures were comparatively low. That the blood chlorides may be

TABLE II  
CHLORIDES OF WHOLE BLOOD IN MISCELLANEOUS CONDITIONS

CASE	AGE	SEX	CHLORIDES AS NaCl	UREA N	SUGAR	PLASMA CO <sub>2</sub>	CONDITION
			per cent	mg. to 100	per cent	c.c. to 100	
1. S.T.	38	♂	0.594 0.538 0.513 0.480 0.394	100 161	0.136	20 20 20	Advanced nephritis, re- stricted chloride diet
2. F.M.	37	♂	0.587	43	0.092		Cardio-nephritis
3. A.W.	8	♀	0.585	91	0.157	27	Acute nephritis
4. M.W.	44	♂	0.550	117	0.136		Diffuse nephritis, edema, death
5. M.D.	30	♂	0.537	43	0.121		Advanced nephritis
6. C.B.	48	♀	0.529	25	0.128		Cardiac decompensation, acute nephritis
7. L.G.	35	♀	0.525	88	0.131		Luetic kidney
8. M.I.	75	♂	0.494	121	0.172		Advanced nephritis
9. R.K.	67	♂	0.456	28	0.142		Prostatic obstruction
10. A.S.	24	♀	0.519	16	0.130	43	Postpartum eclampsia
11. J.Z.	23	♀	0.600	12	0.093		Pernicious anemia
12. J.H.	62	♂	0.519 0.457 0.443	14			Essential hypertension
13. A.K.	40	♀	0.500	12	0.134		Essential hypertension
14. J.M.	45	♂	0.492	13	0.121		" "
15. G.M.	49	♂	0.432	16	0.101		" "
16. E.K.	64	♀	0.478		0.300	44	Diabetes
17. C.J.	50	♀	0.444		0.375		"
18. J.J.	40	♂	0.444		0.440	42	"

readily lowered in some cases of advanced interstitial nephritis by restricting the chlorides in the diet is well brought out by the observations on Case I, this taking place while the nitrogen retention was increasing.

#### GENERAL DISCUSSION

Although the practical value to be derived from the estimation of the blood chlorides can hardly be compared with that of some of the other blood constituents already described, still it is believed that the preliminary estimation of the chloride content of the blood in cases of nephritis may often be of great assistance, particularly in indicating the extent to which chlorides should be restricted in the diet. Furthermore, this estimation should be utilized to determine when the blood chlorides have returned to their normal level. It is believed that in the past, chloride restrictions have often been made when they were not indicated, and, when indicated, have been continued until in some cases the chlorides of the blood reached a subnormal concentration.

In general it may be stated that high blood chlorides have been found in nephritis, certain cardiac conditions, in anemia and some cases of malignancy (possibly due to an accompanying renal involvement), while low values have been observed notably in fevers, diabetes and pneumonia. The chloride retention in most cases of nephritis apparently results from impaired renal function.

The excretion of chlorides and nitrogen seem to be fairly independent functions. In contrast to so-called parenchymatous nephritis, the function of excreting chlorides in interstitial nephritis appears to be much less impaired than that of excreting nitrogen. Consequently a restriction in the chloride intake in the latter condition may fairly quickly restore the chlorides to normal.

When cases of advanced nephritis with marked nitrogen retention are put on a restricted chloride diet it is sometimes noted that the blood chlorides drop to a subnormal level, such as is occasionally found in severe diabetes. A possible explanation for this is that, owing to the large amounts of urea and sugar present in the blood in these conditions, less chloride is needed to maintain normal osmotic conditions. This may also help to explain the increased blood chlorides in anemia.

It is of considerable interest that the chloride retention in pneumonia is associated with a decrease in the chloride concentration of the blood.

#### ESTIMATION OF THE BLOOD CHLORIDES

The estimation of the chloride content of blood is made with the aid of volumetric methods long employed in analytical chemistry. It has simply been necessary for the physiologic chemist to completely remove the blood proteins so that the chlorides could be titrated. When care is employed, it is possible to ash the blood and determine the chlorides in the ash, but such a method is not suited to practical purposes. A number of different methods have been suggested for the precipitation of the proteins (these are discussed elsewhere<sup>1</sup>), but none of these have worked as well in our hands as the use of picric acid first employed by Van Slyke and Donleavy<sup>15</sup> for this purpose. Since picric acid is used for the precipitation of the proteins in the methods for creatinine and sugar estimation already described, it is possible to save considerable time



by utilizing a portion of this same filtrate. (Myers and Short<sup>4</sup> have shown that this 1 to 5 dilution of the blood extracts the chlorides quite as well as the 1 to 20 dilution employed by Austin and Van Slyke.<sup>16</sup>)

Having obtained a blood filtrate suitable for the chloride estimation, one is confronted with the selection of a method of chloride titration. McLean and Van Slyke<sup>17</sup> have suggested an iodimetric method which is delicate and gives a sharp end point when the starch solution is fresh. We are inclined to prefer, however, the well known thiocyanate titration of Volhard, using iron as an indicator. The end point in the titration is possibly not as sharp, but the solutions are permanent, and may be readily prepared by diluting the solutions employed in the Volhard-Harvey<sup>18</sup> method for urine.

*Method.*<sup>4</sup>—The chloride titration may be carried out on the same picric acid filtrate as employed for the estimation of the creatinine and the sugar already described, or the following technic may be employed for the precipitation of the proteins: To 12 c.c. of distilled water in a 20-25 c.c. centrifuge tube are added 3 c.c. of whole blood (or plasma), and then about 0.5 gm. of dry picric acid. The mixture is now stirred until protein precipitation is complete and the mixture turns a bright yellow color. The precipitate is next thrown down in the centrifuge, and the supernatant fluid filtered into a dry tube.

Five c.c. of the filtrate are then pipetted into a centrifuge tube of 25 c.c. capacity and 20 c.c. of the standard silver nitrate-acidified ferric alum indicator solution\* added. The contents are stirred to insure thorough mixing and the silver chloride precipitate thrown down in the centrifuge. The clear supernatant fluid is decanted into a clean dry beaker and 20 c.c. pipetted into a small porcelain evaporating dish for titration.

The titration is made with ammonium thiocyanate solution\*\* of such strength that 1 c.c. is the equivalent of 1 c.c. of the silver indicator solution. The end point is definite and consists of the first permanent tinge of reddish brown which extends throughout the mixture. Some experience may be necessary before the end point is always recognized, but thereafter there need be no difficulty in obtaining exact duplicate titrations. Passing the end point by one drop will introduce an error ordinarily of about 0.5 per cent in estimating chlorides in 100 c.c. of blood.

The calculation may be carried out with the aid of the following formula:

$$20 - \left( \text{titer} \times \frac{5}{4} \right) \times 0.5 \times 100 = \text{mg. of sodium chloride in 100 c.c. of whole blood or plasma.}$$

The 5 c.c. of picric acid filtrate contains the chlorides from 1 c.c. of blood. Since only four-ninths of this is titrated, it is necessary to introduce a correction in the formula. The 20 is the amount of standard silver solution employed, 0.5 the equivalent strength in mg. of NaCl and the 100 the factor required to convert the figures to mg. per 100 c.c.. If the figures are desired in per cent, this may obviously be obtained by moving the decimal point forward three places.

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- <sup>1</sup>McLean: Jour. Exper. Med., 1915, xxii, 212, 366.
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- <sup>5</sup>Schmidt: Charakteristik der epidemischen Cholera, Leipzig und Mitau, 1850.
- <sup>6</sup>Widal and Javal: Semaine méd., 1905, xxv, 313.
- <sup>7</sup>Widal and Javal: La Cure de Déchloruration, Paris, 1906.
- <sup>8</sup>Ambard: Retention chlorurée dans les néphrites interstitielles, Thèse de Paris, 1905.

\*The standard silver nitrate-acidified ferric alum indicator solution may be prepared by dissolving 2.904 gm. silver nitrate in distilled water and making up to 1000 c.c. and then mixing with 1000 c.c. of acidified indicator containing 100 gm. of crystalline ferric ammonium sulphate and 100 c.c. of 25 per cent nitric acid. Two c.c. of this combined solution are the equivalent of 1 mg. of sodium chloride. Both the silver nitrate solution, and the acidified indicator solution are one-tenth (combined one-twentieth) the strength of similar solutions employed in the Volhard-Harvey method for urine.

\*\*The ammonium thiocyanate solution is standardized against the silver nitrate and made of equivalent strength. It contains approximately, 0.65 gm. of the thiocyanate to 1000 c.c. It is one-twentieth of the strength employed for the Volhard-Harvey method in urine.

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<sup>10</sup>Ambard: *Physiologie normale et pathologique des reins*, Paris, ed. 2, 1920.  
<sup>11</sup>Ambard and Weill: *Semaine méd.*, 1912, xxxii, 217.  
<sup>12</sup>Allen: *Jour. Am. Med. Assn.*, 1920, lxxiv, 652.  
<sup>13</sup>Mosenthal and Short: Private communication.  
<sup>14</sup>Host: *Jour. Lab. and Clin. Med.*, 1920, v, 713.  
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<sup>16</sup>Austin and Van Slyke: *Jour. Biol. Chem.*, 1920, xli, 345.  
<sup>17</sup>McLean and Van Slyke: *Jour. Biol. Chem.*, 1915, xxi, 361.  
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# PREPARATION AND STANDARDIZATION OF POLYVALENT ANTI-PNEUMOCOCCIC SERUM\*

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AT THE present time the requirements of the Hygienic Laboratory, Washington, for the standardization of a polyvalent antipneumococcic serum, call for a serum that shall protect white mice against Type I pneumococcus only. In other words, although the horses which are producing the serum are required to be injected with antigens composed of all types of the organism, the relative strength of the antibodies against these other types are not taken into consideration when the final test of the serum is carried out. Nothing is known, therefore, of the value of this sort of a serum except for Type I pneumonia, and its protective value against the other types is questionable. It is no doubt felt, as the reports of Cole and his associates of the Rockefeller Hospital were unfavorable concerning the protection afforded by Type II and III serum, that to require a standard except for Type I would be without significance and valueless.

However, irrespective of these adverse publications, there is a growing and a healthy demand for a polyvalent antipneumococcic serum from sources that must bear recognition. Many physicians find it impractical and even out of the question to submit cases of pneumonia to a test for proper diagnosis as to the type of the organism responsible for the infection, and are not only willing but anxious to use a polyvalent serum. Also, to require a case to wait from eight to twenty-four hours for this diagnosis greatly diminishes the chances of the patient's recovery, and it ought not to be necessary to take this chance.

In fulfilling the requirements for this class of sera, an attempt should be made to produce a serum as potent as possible and one that shall protect mice against all types of the organism in high dilutions, so that some sort of a standard can be adhered to. This is not only a protection for the physician as well as the patient, but, also, a means of checking the results following the administration of the serum, for statistical purposes. At the present time, the reports as regards the clinical value of Type II and III sera are entirely too meagre and there has been no report following the use of a polyvalent serum standardized against all types. In fact, relatively little has been done or at least published concerning the preparation and standardization of sera except for Type I.

According to Dochez and Avery, the groups of the pneumococcus vary in their pathogenicity for human beings, and he gives the order of their virulence as follows: Group III, Group II, Group I, and Group IV; Group III being the most virulent. They state, also, that the degree of protective power developed in the sera of animals immunized against members of the different groups varies inversely with the virulence and with the amount of capsular development. By using 0.5 c.c. of serum and 0.1 c.c. of a bacterial suspension, Cole reported, of Type I serum, a protection against one hundred thousand fatal doses and, of Type II serum, Avery reported, a protection against ten thousand fatal doses.

\*From the Research Laboratory, L. J. Burke, Davis & Company, Detroit, Michigan.  
Read before the Society of American Bacteriologists, Boston, Mass., December 29, 1919.

The figures are not given as regards Type III serum, although it was said to be less than Type II, and nothing has been published as regards a polyvalent serum.

With horses immunized against single groups or types, the authors have been able to produce sera of the following strengths: Type I, a protection against ten million M.F.D.; Type II, a protection against ten thousand M.F.D.; Type III, a protection against ten million M.F.D.; and of those strains of Type IV, a protection against ten million M.F.D. This does not quite agree with the results of Cole, as his protection against Type III was extremely low.

With individual horses immunized against all types the authors have produced polyvalent antipneumococcic sera showing a uniform protection against all types as follows: Type I, a protection against ten million M.F.D.; Type II, a protection against one hundred thousand M.F.D.; Type III, ten million M.F.D.; and of those strains of Type IV used, a protection against ten million M.F.D.

As far as laboratory animals are concerned, therefor, a sufficient protection with a polyvalent serum was obtained by the authors against Type I, II and Type III and for those strains of Type IV which were used as antigens in immunizing the horses, and the serums producing this protection have been standardized against all types, using the method required at the present time by the Hygienic Laboratory for Type I serum.

In producing this polyvalent serum, horses already giving a high titre of Type I serum were chosen and then injected with mixed antigens composed of Type II, III, and IV, using the regular schedule of injections as recommended for Type I. In carrying on this work twelve horses were under treatment. The following chart gives the results of the tests of the sera of the various horses.

#### STANDARDIZATION OF POLYVALENT ANTIPNEUMOCOCCIC SERA

Figures represent number of minimum fatal doses of the pneumococcus against which 0.2 c.c. of serum will protect; white mice being used for test animal, as required by the government.

At the present time it is required that 0.2 c.c. of Type I serum will protect against 10,000,000 minimum fatal doses.

SERA	ANTIGENS			
	TYPE I	TYPE II	TYPE III	TYPE IV
<i>Type I</i>				
Horse 1201	10,000,000		1,000,000	
" 1184	10,000,000		1,000,000	
<i>Type II</i>				
Horse 1203		10,000	1	
" 1204		1,000		
<i>Type III</i>				
Horse 1205			10,000,000	
" 1206			10,000,000	
<i>Type IV</i>				
Horse 1211				10,000,000
" 1212				10,000,000
<i>Polyvalent</i>				
Horse 285	10,000,000	10,000	10,000,000	
" 385	10,000,000	100,000	10,000,000	10,000,000
" 859	10,000,000	100,000	10,000,000	
" 909	10,000,000	10,000	10,000,000	

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## SOME PERSONAL EXPERIENCES WITH EPIDEMIC RESPIRATORY DISEASES IN THE ARMY, WITH SOME REMARKS ON METHODS OF CONTROL\*

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ARMY medical officers are greatly concerned over the prevalence of respiratory disease in the army in 1917-18 and the great epidemics of that period have provided a copious literature on the diseases themselves, the pathology, clinical findings, complications, mortality, sequelae, etc., though little has been written concerning practical measures to control these diseases. It must be admitted that, to a certain extent, when young individuals of military age are brought together, these diseases are an inevitable accompaniment and one draws this impression from reading of the epidemics, as well as from the conclusions of the epidemiologists who have studied them. This article is written from the side of experience, not in the great epidemics of the army camps, but in smaller epidemics, with the special purpose of drawing some deductions from these smaller epidemics which seem to point to great principles of epidemic control which are applicable to larger epidemics. I have a belief that there are certain principles which are immutable to a considerable degree and which will help to prevent disease that is of contact origin, such as the diseases of our respiratory epidemics, and it is in the hope that these principles may be brought out that this is written.

The diseases to be considered are those for which we have no specific protective agents and it is only these which can be truly dangerous. Those which may be guarded against by specific inoculation may appear, but they will never be widespread if the command is even partially protected. It is those diseases too, for which we have no specific protection, which must be controlled if possible to do so, largely, because of that fact. It would be folly to neglect the water supply if we had no typhoid vaccine, on the theory that we had no specific immunizing agent and therefore, could do little to prevent the disease. It seems to me equally dangerous to consider that the natural susceptibility to measles of the southern recruit who has hookworm makes it inevitable that he must have measles if he comes into an army camp. This is far-fetched, it is true, but should be thoroughly realized in order that proper precaution be taken. The real danger lies in the fact that if one considers respiratory disease as inevitable, one is unconsciously going to do little of real good to prevent his men from getting it, while as a matter of fact and of experience, I thoroughly believe that there is much of practical value that can be done to prevent such infections, even if there is no specific aid to protection. Every effort, of course, must be made to discover new specific protective substances, but while waiting for these, one

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should not neglect any part of possible protective work which has done good in experience.

In presenting these practical measures which have proved successful, I shall discuss each little epidemic I have studied personally, bringing out those points which seem to me important and then concluding with the summary which governs epidemic control in general. If it can be shown that these measures which have been used have produced results which remain, then there may be as much argumentation as desired against them as theories or principles, but the results will not have been changed and can never be attacked.

#### MEASLES

During the early part of 1917, before our country declared war, the recruiting service of the army was carried on at the recruit depots. A number of young men, seeing that war would come, did not wait to be called, but came into the army at once. This increased the work of the recruit depots and congested them to some extent. I was on duty at one of these depots, Fort McDowell, California. Some time in December, 1916, or January, 1917, cases of measles and scarlet fever began to appear, some from detachments received from Eastern depots and some from the country about. Fairly soon, a small epidemic had started among the susceptible timber. I have no adequate statistics for this entire period and it would be difficult for me to secure them now because a good many of these cases were transferred to another station for hospital treatment. It became necessary to open a hospital at the depot for the care of these infectious cases and I have figures for the period from February 22 to September, 1917. German measles, mumps, chicken-pox and scarlet fever were present at the same time. The following table shows the measles figures. The strength of the command varied from 2000 to 3500.

	<i>Measles</i>
Admitted	185
Died at Depot	6
Transferred because of complications	9
Other complications at depot:	
Bronchopneumonia	5
Lobar pneumonia	3
Otitis Media	2
Mastoiditis	1
Pleurisy	1

#### OTHER CONDITIONS ADMITTED SAME PERIOD:

German measles	19				
Bronchopneumonia	6	Transferred	0	Died at Depot	1
Lobar pneumonia	7	Transferred	5	Died at Depot	1
Pleurisy	1				

It is not known how many of these were in patients who had had measles.

The figures show that a real epidemic was present. It is known that during a part of this time recruits were freely sent out to stations and that many cases of measles developed from those at the depot. The usual period for a recruit to be held for training was three to four weeks and a constantly changing recruit population was present. The element of susceptible material was therefore, always present. For a considerable time, until some time in April, there was no attempt to segregate the new recruits, but these were assigned to recruit

companies in barracks from which measles cases were being admitted. There was also no attempt made to get all the measles cases out of the companies, i. e., the recruits were expected to present themselves at sick-call. There were no attempts to get the cases in their preeruptive stage. For those conditions I was not responsible. Up to April, the epidemic was steadily increasing as new susceptible material was added to the infected companies. In April decided changes were made. These were chiefly:

The new recruits were sent to another camp away from the infected companies.

All recruits were inspected daily to pick out sick men.

All men with colds were isolated.

Infected companies were not allowed to send recruits away.

Immediate contacts of cases were isolated.

Bedding, etc., of cases was disinfected.

General instruction of the command was carried out.

The effect of the simple measures noted above was almost instantaneous. Secondary cases continued to come in from the men already infected but the new recruits remained practically free and the infected companies rapidly became free from measles. It is interesting to note that this was the first recruit depot to secure female nurses for the care of the sick and in a short time the contagious cases became practically extinct, so much so that the nurses complained that they were not needed. I could show this reduction in measles by chart but I believe it is not needed; the data is on file in the sanitary reports from the depot for that period. During all this period there was no interruption of recruiting, no quarantine, no proscription of amusements, etc.

Measles has for some time been recognized in the army as a serious disease, especially where recruits have congregated. It was never serious in ordinary commands, though measles did occur in these commands sporadically. In 1911, Christie<sup>1</sup> reported an epidemic at Columbus Barracks, another recruit depot, of 183 cases and called attention to the severe pneumonia and other complications. He called especial attention to the necessity of getting the cases early by inspection and by isolating all cases of colds. In 1912, Kilbourne<sup>2</sup> reported a series of 600 cases from the same depot, elaborating Christie's report. As for older history, it is stated that there were in the Union Army, during the Civil War, 75,177 cases with 5,174 deaths and that in the Confederate Army the disease became so dangerous that regiments had to be disbanded and sent home.<sup>3</sup>

There is a strong sentiment in the medical profession to the effect that one might as well allow troops to have measles because "they are going to get them anyway, and you may just as well let them have measles in this country and avoid getting it on the transports and at the front." This, I believe, is a fallacy. There is no doubt that the men are susceptible, but that does not mean that they must have it while in the army, or that, even if some of them get it they must all have it at the same time. Measles, when it occurs sporadically, is not dangerous, and it is only when a large number of cases occur together, i. e., under epidemic conditions that the fatality becomes great and the condition serious. It is the "secondary invaders," the streptococci, the pneumococci and other organisms which are present and which take on increased virulence in the presence of measles that

give us the "virulent measles" and cause us to lose so many young men in camp. If we keep the men from having an explosive epidemic, or an epidemic which picks up increasing numbers of cases as it goes along, we can control it and can allow the men to become immunized in small lots with comparatively little danger. This is not a thing to be done by allowing them to get measles, as is often suggested, but by doing our utmost to control the measles which we are bound to get anyway. Measles is not easily controlled; it is one of the most contagious diseases, but in my opinion it can be controlled by the application of the proper principles, as outlined in the report of the epidemic at Fort McDowell. That epidemic was in no sense necessary and could at least have been greatly mitigated, if not prevented entirely. The epidemics are insidious affairs and to the inexperienced epidemiologist, measles when it begins is not serious. This is the period when the mischief is done. Cases are allowed to go on infecting the command until they accumulate in such numbers that they become "virulent" at the time of the septic infections. Then the epidemic is really recognized and but little can be done to save lives.

#### SCARLET FEVER

During the period of the epidemic at Fort McDowell, scarlet fever appeared at the same time as measles. For the same period for which I have records, February 22 to September, scarlet fever was an important disease as shown by the following:

Cases admitted:	88
Died at depot	7
Transferred because of complications	20
Other complications at Depot:	
Lobar pneumonia	3
Bronchopneumonia	2
Acute nephritis (fatal)	1
Septicemia (fatal)	1
Otitis media	1
Adenitis	1

The occurrence of German measles at the same time made the differential diagnosis at first difficult. On careful study, it was not difficult to separate them. Two medical officers and several medical department men contracted scarlet fever in the wards. There were a few cross infections as our isolation hospital was not well run until the female nurses arrived, but there was no trouble in controlling the epidemic among the recruits. This disease is much easier to control than measles, partly because of the shorter incubation period and partly because of the lessened contagiousness. The general measures applied to control measles were equally applicable to scarlet fever and the results were evident earlier. A careful study of the cases was made to trace the infection from case to case, and frequently, this could be established in the men sleeping close to previous cases in barracks. No case could be shown to have occurred from outside contact, i. e., there was better evidence to show that it came from the men in the same squad room. For this reason, when a case was taken from barracks, the nearest neighbors were removed to isolation and held over the incubation period. Some cases were taken from these contacts, showing the value of the method.



The value of inspections to determine the sick men in scarlet fever is striking. Unless one has done this, one must be very much surprised to take cases from the companies, well-developed in the disease with the eruption full blown. Were one to wait for sick-call to bring those men into hospital, some of them would undoubtedly never come. They would be "missed cases." During the war, scarlet fever has not proved a very dangerous disease. During 1917, 2,133<sup>1</sup> cases were admitted for the whole army. During 1918<sup>2</sup> there were 8,117 cases. A few camps had admission rates much above the average and it is these camps which furnished most of the cases and naturally most of the deaths. There were 314 deaths from this disease for the two years and these deaths are a reflection upon medicine. We have no specific immunizing agent against scarlet fever, yet it seems that scarlet fever deaths should be largely prevented. The deaths alone are not a true index of the danger of this disease for the number of late fatalities is large, due to nephritis, itself due to the streptococcus. Here, too, the streptococcus becomes more dangerous when scarlet fever cases become so many as to form an epidemic.

#### DIPHTHERIA

Diphtheria is often a real trial in hospitals and other institutions. It was not of especially great importance in the army during the War. In 1918<sup>3</sup> there were 6,947 cases with 123 deaths. During my service at U. S. A. General Hospital No. 24, an abortive epidemic appeared which at first bid fair to be serious. It was easily controlled however, by intensive methods. The hospital population was about 1100.

On February 21, 1919, three cases appeared in two different wards. The next day another case developed in a different ward. The next day, two cases came from the medical detachment. These six cases coming from such wide sources showed at once that there was a source of infection which had been spread throughout the hospital. Only seven cases in all developed, however. I attribute this small number of cases largely to prompt action to prevent spread. The preventive measures taken were:

An immediate conference of department heads with the division of the work to be done among the conference group.

Quarantine of the hospital against the community to protect the latter.

Culturing of all contacts for the detection of carriers. Fifteen carriers were found.

Quarantine of individual wards.

Throat inspection twice daily of all contacts with spraying with 1 per cent phenol with iodine, camphor and menthol.

Investigation of the milk and ice cream supplies.

Antiseptic details for bedding, clothing, etc., of infected cases.

At U. S. A. General Hospital No. 28, Fort Sheridan, Illinois, routine culturing of the throats of all patients admitted to hospital has effectually prevented any indication of an epidemic. Carriers are found from time to time and segregated. An occasional clinical case of diphtheria is found, but there has been no group of cases, as far as I have been able to find.

## INFLUENZA

Epidemic influenza appeared at Fort Sheridan on January 12, 1920. This was the same date as marked the beginning of the epidemic at Great Lakes Naval Training Station and also the epidemic in Chicago. It had been looked for by the Commanding Officer, Col. W. N. Bispham, M. C., who had laid out the procedure to be employed in case of its occurrence. The plans to be followed had been used by him in the epidemic of 1918 at Camp Greenleaf, Ga., and had proved successful there. The steps to be taken were: First: no quarantine. Second: all persons in the garrison were to be inspected daily for signs of respiratory infection. Third: all cases showing any evidence of respiratory infection were to be removed at once to wards where the patients could be observed and the influenza cases selected. Fourth: all influenza cases were to be removed from "suspect" wards and placed in "influenza" wards. Fifth: all pneumonia cases were to be segregated in "pneumonia" wards. The plan worked very well and, in the opinion of the Commanding Officer, that of myself and that of many other persons, the prompt and systematic sorting out of the command did a great deal to reduce incidence and mortality from this disease.

The epidemic began on January 12th with the development of twelve sick men diagnosed influenza in one of the surgical wards. These men were isolated at once in a newly opened ward equipped for isolation with cubicles, etc. The next day a number of other cases were found and from then on the epidemic was typical. An attempt was made to get the cases as soon as they could be found and this was generally successful, for very few came in late in the disease. The great majority were early cases. The peak was reached on January 18th, though it really should have been on the 15th or 16th, this delay was due to failure in getting the sick into hospital promptly. By January 31st, the epidemic was over, being followed by straggling cases and by atypical cases in which the streptococcus played a large part.

Cases were admitted from all departments and all wards of the hospital and from all detachments of the garrison, showing the infection was widespread and that there was opportunity for a large incidence. The patients in the majority, were old surgical cases in more or less debilitated condition and were of the age-group that appears to be most susceptible to the disease. A census of the hospital personnel showed that in the hospital itself, of 3352 persons, 552, or 16.5 per cent had had influenza in the epidemic of 1918. This left the large majority of them susceptible, presumably. In addition to the hospital personnel there was a regiment of line troops in the post, numbering 1300, for which the percentage of susceptibles could not be obtained, and a considerable number of civilians. The entire command is estimated at 5000. Of this number, 522 were admitted as influenza suspects and 368 were diagnosed influenza. The remainder were simple coryzas, tonsillitis, surgical conditions, simple febrile conditions for only part of a day, etc., and some were possibly mild influenzas in which the diagnosis could not be made. All cases of three-day fever or even shorter fever that were sharp infections and otherwise unexplained were called influenza. The incidence of influenza in the command was 1 in 13.6. There were in the cases admitted during the epidemic period, 45 cases of pneumonia complicating

influenza, an incidence of 1 in 111.1 of the command and an influenza case incidence of 1 in 8.1 or 12.2 per cent. There were seven deaths of cases admitted during the influenza period. The case mortality for influenzal pneumonia was 15.5 per cent.

It is believed that the low incidence rate, the low pneumonia rate, and the low death rate are largely due to the plan which had been prepared by the Commanding Officer beforehand and successfully carried out in the hospital, where the troops were under military control and where the plan could be thoroughly carried out. Some of the deaths were in civilians and in cases admitted late in the disease. If all cases could have been secured at the onset of the disease, the mortality might have been still lower. Such a system could not be used in a civilian community without material modification, except, perhaps in industrial communities where medical attention is provided by the industry, or in institutions where there is proper discipline and sufficient skilled help. The conditions in communities are different, however, because the people are much more scattered, the age-groups are diverse and susceptibles are not so closely herded, and the susceptibles are not subjected to the same danger of infection. The separation which normally obtains when sick stay at home must be much less dangerous to the patients, than the close association of the sick men in wards as is necessary in the army, because the secondary invaders are undoubtedly rather universally transmitted from man to man in such close contact in the wards. This has been shown by various observers in the Army Camps. The cubicles do much to prevent this but the enforcement of cubicle isolation is a tremendous task, even in the army. The enforcement of ten days' rest in bed after the fever has returned to normal in the influenza patients required by the Surgeon General has undoubtedly been a large factor in the prevention of secondary pneumonia.

Many persons have the idea that, lacking specific means of protection against influenza, little or nothing can be done to limit its spread. This, I believe is a serious fallacy and the experience of Fort Sheridan, in the epidemic of 1920 should have some positive value in eradicating this idea. Because a person is susceptible, it does not follow that he must have the disease in an epidemic, because he may miss the infection. The Command at Fort Sheridan was largely susceptible, as shown by the census, yet only 1 in 13.6 of the persons of the command took the disease. In order to be effective, however, measures to limit the spread of the epidemic should be taken at once the epidemic appears, because it is probable that the first cases of the epidemic have the most virulent and most infective organisms in large numbers in their respiratory tracts. If the cases that have these particularly dangerous organisms can be isolated, at once, the danger of spread from those persons is greatly minimized. Measures taken several days later, no matter how rigid, will probably be much less effective than early measures which are more simple, but which operate to lock up the first dangerous cases.

The question of quarantine in epidemic influenza is an interesting one. It was largely used in 1918, in some cases with some success, and was used in some places in 1920. It was not used at Fort Sheridan because in this disease it appears to be practically useless and illogical. Even the laity have come to realize the inconsistency of locking up the patients of a hospital when influenza

is present in a community while allowing the attendant staff to come and go. If the community has the disease, why lock up the command if the command has the disease also? Internal quarantine, except for the actually sick is likewise useless. The important thing is to get the sick away from the well and then the well may take advantage of their ordinary duties and pursuits without the worry of any unnecessary hardship in quarantine. Inspections, even in a civil community, I believe are more than practical. Schools, churches, factories, theatres, business offices, and possibly other places could use this method if there were trained personnel available.

#### GENERAL COMMENT

The subject of epidemiology is one of which much will be heard in later years. Little has so far been written of the science of this study and this is particularly so of the epidemics of respiratory infections or diseases. Epidemiologists have for some years been at work in State Boards of Health, in which perhaps their greatest work has been done in connection with the intestinal group of diseases, typhoid, paratyphoid, dysentery and food-poisoning. In the tropics and in Eastern countries, cholera has claimed a large share of their work. In the Far East, plague has been studied and practically controlled and in this country also, plague has been controlled by epidemiologic methods. Meningitis and poliomyelitis are two diseases which are characterized by epidemics which have been handled by epidemiologic methods. Typhus fever, yellow fever, and malaria, insect-borne diseases, have yielded in great measure to epidemiology, and trench fever may be classed in the same group. Tuberculosis, though not strictly an epidemic disease has received a vast amount of study but without brilliant results.

The diseases which have been so dangerous in the army during the war, measles, scarlet fever, influenza and pneumonia have not received, in the past, as much attention from epidemiologists as those other diseases and two others might be mentioned which are potent sources of trouble in bodies of individuals, mumps and whooping cough. Pneumonia has been studied and controlled while in epidemic form by General Gorgas in Panama and the same authority has extended his work over the pneumonia of the diamond mines of Africa. These diseases have not been so marked by epidemics in civil life as in armies, except, of course, influenza, which never before, probably, had so large an army in which to spread as was provided for it in this war.

The influence of concentration of recruits during war periods on the incidence and death rates for respiratory diseases is shown by the following figures for measles.

Munson	PERIOD	ADMISSIONS PER 1000	DEATHS PER THOUSAND
	Civil War	31.72	2.02
	1868-84	1.88	0.004
	1885-94	4.85	0.004
	1895-98	13.72	.69
Report of Surgeon General 1914	1917	87.08	1.70
	1918	29.34	.87

Munson makes this comment: "During 1898, when the proportionate number of recruits was great, the rate admission was by far the highest during June and July—thus showing the greater importance of length of service as compared with season in influencing the prevalence of this disease. During the same year, the admission was 19.82 per thousand soldiers stationed within the United States, while there was a total rate of 48.25 for the troops serving in the West Indies and the islands of the Pacific."

Pneumonia can also be shown to be much more prevalent during war periods than during peace times. During the Civil War, the pneumonia admission rate was 32.45 per 1000 and the death rate 7.79 per 1000. For the period 1868-84 the admission rate was 6.55 per 1000 and the death rate 1.01 per 1000. During 1898, the admission rate was 4.84 and the death rate .83, while in 1897, the preceding year, the admission rate had been 2.9 and the death rate .21.

The death rate in 1862 for all acute respiratory diseases was 8.40 per 1000. After the year 1866 no death rate for total respiratory diseases reached a point of over 2 per 1000 until the year 1918. The rate reported by the Surgeon General for this year is 15.75 per 1000. This again shows the influence of war periods upon the rates of sickness and death for respiratory disease, due to the aggregations of individuals into large units in close association.

When we study individual camps or garrisons, we will find that where relatively large numbers of recruits are closely associated for periods of time, the rates for respiratory disease are raised because of epidemics of such diseases as measles, accompanied by pneumonia, and this is well shown at the recruit depots, as reported in the beginning of the paper under measles. The epidemics at Columbus Barracks and at Fort McDowell were accompanied by similar epidemics at the other depots.

It is particularly in the army and navy that epidemics of the common respiratory diseases occur, and it is in the army and navy that epidemiology has its best field to work in the study and control of these diseases. When mobilizations of troops occur, epidemics of these diseases must be expected, and when they do occur, they require the services of a well-informed epidemiologist in each camp to carry out the measures that are of service. Since such epidemics do not often occur in civilian communities, civilian physicians are not trained in their control, and the medical officers who are called upon to manage them, should either be from the army, trained in the history of these diseases and in methods of attack and prevention, or they should be from civilian life and trained in the army or other military branch. One of the qualifications needed in such a medical office is that the physician should have had actual experience in one or more of the epidemics in army service. This qualification could now be fulfilled by large numbers of physicians and if another war is thrust upon us within a short time, they can be easily obtained. It should be provided, however, that when the next war comes, whether it be soon or late, that such men be ready.

The incidence of respiratory disease in the army is largely among the recruits. Men who have had a year or more of service have a far lower incidence and mortality rate than the recruits of short service. If the youths of the Nation could be called into camp regularly and trained in military duties, the next

war would find them highly seasoned and our fear of the respiratory infections would be very much reduced. It is to be hoped that such a beneficent system will some day be provided for our boys, though there is little to convince us that our legislators will see the truth of this matter. The fact that so many recruits become sick with these diseases has convinced a great many otherwise careful people that the diseases are inevitable accompaniments of service, and therefore, the sooner the recruits become seasoned, the better. There is a great practical difference, however, between explosive seasoning and gradual seasoning. During peace times, the army regularly absorbs a number of recruits who are distributed to seasoned organizations. They have their epidemics at the depots where the new men are assembled, but the effect upon the total sickness of the army is not large. They continue to have their infections after reaching their organizations, but large epidemics among these seasoned troops are rare. Therefore, the recruits are assimilated without great effect upon death rates. When the recruits are thrown together in great numbers, as was done in our camps, unavoidably, during the war, explosive outbreaks occur which do great damage to lives. If we are convinced that these diseases are inevitable and cannot be prevented, and that anyway, it is a good thing to get over with, we will not be alert to the danger and will do little to prevent it. This is not necessary hardening but is a grave risk and should never be countenanced. Although we have no specific means of prevention, it is my opinion that practical measures of inspection and isolation of the sick will do a great deal to delay the exposures of the men and to reduce the death rate.

The experience of the past war has shown the greater susceptibility to measles and other disease of rural recruits over the city recruits. This is not a new observation. Munson quotes Woodhull as follows: "The agricultural recruit will be better nourished and at first may appear the more vigorous, due to his previous life of moderate exercise in the open air, uninterrupted nightly sleep, and of stated and sufficient meals. \* \* \* On the other hand, the young man from the city has been accustomed to all grades of physical and mental excitement, he has probably eaten spare, irregular and poorly cooked meals, and has lived in crowded and ill-ventilated rooms. He may have been insufficiently clothed, and has certainly been used to late and irregular hours and to spasmodic physical exercise. \* \* \* After elimination by length of service the country regiments rival those from the city in endurance, and generally excel them in the familiarity in the ways and with the implements of outdoor life." The discovery that rural recruits are more susceptible to measles does not aid in the solution of the epidemic problem because these recruits must be accommodated and trained and their health must be safe-guarded. This subject points out the necessity that medical officers responsible for the prevention of the epidemic respiratory diseases must be acquainted with the problems of military service and with military hygiene in history.

There was one argument during the war which was propounded against aggressive work against respiratory diseases which was hard to meet. This was that unless troops had developed measles and kindred infections in the camps that they would get them in the front areas in France and there be much more of a danger and nuisance than they would be in the camps at home. This was a

fallacious argument and can now be shown to have been such. The records of the Expeditionary Force in France show that the incidence of influenza in the troops in France was considerably less than at home, although the strength of the armies was about the same. "During the last four months of 1918 deaths from influenza and pneumonia number 22,186 in the United States and 8,842 in the American Expeditionary Forces, France, the respective commands being of approximately equal strength."<sup>5</sup> This may be readily explained by reason of the fact that the troops in the front areas were much more widely scattered in billets, rest areas, small towns, and in advance formation than the troops at home in the large camps. Separation was here a marked factor. In the large camps of the Service of Supply, the groupings were greater and the incidence of influenza relatively higher, but even on the crowded transports the mortality rates were not higher than those of the camps at home. The old argument, therefore, that we should allow the recruits to have measles by purpose or by half-hearted efforts to control the disease in camps should forever be forgotten.

#### PRINCIPLES OF EPIDEMIC CONTROL

That there are principles of epidemic control which should govern our action in general must be evident to most of us. There must also be general principles for the control of epidemics of respiratory diseases which should govern for all epidemics whether these are great or small. We may go farther and say that when these principles are applied effectively, in time, epidemics should tend to be small. There are, doubtless, conditions under which disease has become so overwhelming as to make control impossible, but even then there are principles of broad nature which may be applied with positively effective results. It must be acknowledged that if we were to do nothing when a major epidemic of influenza rushes in, conditions would be much worse than they were in any of the army camps during the influenza epidemic of 1918. Medical officers did a great deal to meet those strenuous conditions and no one can say that those services did no good in reducing mortality and probably incidence in that epidemic. If we were to analyze the conditions in different camps, it could probably be shown that some camps had good results with relatively low incidence and mortality, while other camps had high incidence and high mortality. This disease is such a universal one that local conditions in the resistance of the individuals or local conditions in the camps can have played a small part, and it is very probable that the action taken by camp authorities did play an important part in the showing made by the particular camps. Whatever was done that was effective must have been done under certain great principles, whether intentionally or not, as explained by the greater principle of cause and effect.

The determination of the principles that should govern our action in epidemic control will prove a fruitful field for investigation and improvement in public health work in the military and in civilian life. Without a careful study of them no medical officer will be competent to deal with epidemic conditions and without a thorough acquaintance, no physician can well avoid doing much that is useless and careless. Epidemic work calls for the application of the most careful detail possible according to the great principles that have been shown to be logical and helpful. Soper<sup>6</sup> has done much to make these clear and it is to be

hoped that there will be more and more attention paid to this aspect of medical instruction in the medical schools, in the army, and in boards of health. I have used my own experience with the help of Soper's principles to attempt to put down on paper some of these principles which seem to be sound and of great aid to the practical epidemiologist. These will not be elaborated because they have been pointed out in the substance of this report.

1. Organization and preparation for epidemics should be done beforehand, in as great detail as possible, so that it may be ready for application when the epidemic begins.

2. Epidemics are easy to control when the disease can be attacked at its beginning, but when it has already widely spread are extremely difficult to control.

3. Diseased persons are the great sources of danger in epidemics; things are not generally to be feared.

4. Direct and obvious channels of infection are much more dangerous than long, roundabout and mysterious ones.

5. Sources of infection must be searched for and not awaited.

6. The early diagnosis of sick persons is necessary to prevent disease spread.

7. In the military service, inspection of healthy persons is necessary to detect early disease.

8. Early and strict isolation of possibly infected persons is essential to control the disease.

9. Early action against disease is much more potent than action, no matter how thorough, at a later time.

10. Education of individuals enables them to assist in disease prevention.

11. Separation of individuals lessens disease incidence.

12. Crowding of individuals contributes to disease incidence.

13. Proper methods improperly controlled are not effective.

14. Proven methods of artificial immunization are to be used.

15. Lack of specific immunizing agents for specific diseases does not make epidemic control hopeless. Such a lack makes it imperative that every practical method be most thoroughly applied.

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<sup>3</sup>Mumson, E. L.: *Military Hygiene*, 1901.

<sup>4</sup>Report of the Surgeon General, U. S. Army, 1918.

<sup>5</sup>Report of the Surgeon General, U. S. Army, 1919.

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# LABORATORY METHODS

## MODIFICATIONS OF VAN SLYKE'S TITRATION METHOD FOR ESTIMATING THE ALKALI RESERVE OF BLOOD\*

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VAN SLYKE'S alkali reserve titration method<sup>1</sup> appealed to us at once as a simple way of detecting acidosis and of determining its severity. No expensive apparatus is required and the technic is easier than that of Van Slyke's original method.<sup>2</sup>

Classroom results, however, were disappointing. The error was due chiefly to the difficulty in matching a turbid plasma mixture with a perfectly clear phosphate standard and to the feeling of uncertainty as to the correct end-point. A few of the students obtained results that were sufficiently accurate for clinical purposes, but most of them were dissatisfied with the method because of this annoying uncertainty.

In order to overcome this difficulty we have prepared a turbid standard. With the modifications suggested below, the method now gives complete satisfaction, and the results are practically identical with those secured by the use of Van Slyke's apparatus.

### MODIFICATIONS OF VAN SLYKE'S METHOD

1. *Indicator.*—Easier matching is secured by using one half quantity of neutral red, (0.3 c.c. of 0.05 per cent solution instead of 0.1 per cent). Dissolve 50 mg. of powdered neutral red<sup>3</sup> in 100 c.c. of 50 per cent alcohol, and keep the solution in a nonsol flask that has been painted black.

2. *Flasks.*—We found the 50 c.c. flasks too small for convenient mixing and color matching, and, therefore, we use 120 c.c. nonsol Erlenmeyer flasks.

3. *Preservation of the Standard Alkali.*—One of the most important modifications consisted in dividing our supply of  $\frac{N}{50}$  sodium hydroxide between quite a number of small nonsol flasks. Ordinary glass changes the alkalinity of the solution in a short time. Exposure of the solution to the air, as is necessary in filling the burette, leads to the formation of carbonate, which interferes with the end-point and results in an increased titration. We found that generally we could use the solution from one flask only for one day, the titration value being about 0.07 c.c. greater when used later.

4. *Permanent Color Standards.*—Two of these are needed, one that is perfectly clear (used only for checking the  $\frac{N}{50}$  solutions) and one that is

\*From the Department of Biochemistry, Medical School, University of Oregon.

†We used Grubler's. Some samples of neutral red that we have tested were found to be worthless.

turbid. A phosphate mixture ( $P_H$  7.4) may be used for the clear standard, as Van Slyke directs. Since this standard contains neutral red it will not keep more than a few hours, so that it must be prepared fresh each day. We prefer, therefore, to use for both standards a mixture that has a permanent color. This was suggested by the previous work of one of us in devising permanent standards for determining the  $P_H$  of urine.<sup>3</sup>

The turbid standard differs from the clear standard only in containing raw starch (which proved to be more satisfactory than inorganic insoluble substances). Starch cannot be used with neutral red and the phosphate mixture of Van Slyke's standard. The permanent standards are prepared as follows:

To 60 c.c. of special buffer phosphate solution ( $P_H$  6.8) add 5.6 c.c. of amaranth\* solution (dissolve 8 mg. in 100 c.c. distilled water and add 0.5 c.c. chloroformthymol preservative), and 5.2 c.c. of paranitrophenol\* solution (dissolve 20 mg. in 10 c.c. alcohol and dilute with 90 c.c. distilled water.) The amaranth, paranitrophenol and buffer phosphate† solutions are the same as those used in the preparation of the standards for estimating the  $P_H$  of urine.<sup>3</sup> Transfer 30 c.c. of the mixture to each of two nonsol Erlenmeyer flasks (120 c.c.) and add 0.2 c.c. of chloroformthymol preservative to each. Keep one of these as the clear standard. To the other add 20 mg. of dry finely powdered corn starch (that used for cooking is quite satisfactory). This is the permanent turbid standard. Cork both flasks tightly and seal with paraffin. They keep indefinitely if protected from strong sunlight.

The error involved in the use of the clear standard for plasma titrations is illustrated in Table I.

TABLE I

COMPARISON OF ALKALI RESERVE ESTIMATIONS USING CLEAR AND TURBID STANDARDS

PLASMA	TITRATION METHOD				ALKALI RESERVE FIGURE BY VAN SLYKE'S APPA- RATUS
	N 50 HCl NEUTRALIZED		ALKALI RESERVED FIGURE		
	USING CLEAR STANDARD C.C.	USING TURBID STANDARD C.C.	USING CLEAR STANDARD	USING TURBID STANDARD	
1	3.35	3.23	75.0	72.3	71.6
2	2.65	2.45	59.3	54.8	54.4
3	2.43	2.25	54.4	50.4	50.6
4	2.30	2.15	51.5	48.2	48.1
5	1.55	1.44	34.7	31.4	30.8

## TECHNIC OF TITRATING THE ALKALI RESERVE OF PLASMA

Draw the blood at least three hours after the last meal and after a one hour rest period. Have a paraffined centrifuge tube ready, containing 0.07 c.c. of 39 per cent potassium oxalate solution and about 1 c.c. of paraffin oil. After inserting the needle into the vein release the ligature before letting blood into the syringe. If duplicate estimations are desired, draw 10 c.c., otherwise 5 c.c. is sufficient. Deliver the blood at once below the oil, tilt and rotate the tube to ensure quick mixing (do not shake). Keep the blood cold, centrifuge as soon as possible and draw off the perfectly clear plasma with a pipette.

\*We secure these chemicals from Emmer and Amend.

†This may be prepared from Merck's special anhydrous phosphates ("Sorensen's") which are the most reliable on the market. Dissolve 1.362 gm.  $KH_2P^+O_4$  and 1.422 gm.  $Na_2H^+P^-O_4$  in about 80 c.c. distilled water and dilute to 100 c.c.

Measure accurately 2 c.c.\* of plasma and 5 c.c.\*  $\frac{N}{50}$  HCl into a medium-sized Florence flask. Add a drop of caprylic alcohol and rotate the flask for at least one minute in order to spread the plasma mixture in a thin film on the wall. Pour the mixture into a 120 c.c. Erlenmeyer flask and wash the rest into it with 20 c.c. of distilled water (3 rinsings). Add 0.3 c.c. of the neutral red solution, and titrate with carbonate-free  $\frac{N}{50}$  NaOH to the proper end-point. Keep the top of the burette\* covered with a test tube. The burette must have a fine tip so as to deliver about 0.02 c.c. with each drop.

The end-point is determined by comparing the plasma mixture with the turbid standard (well mixed). The color matching is easy even when the standard has a different degree of turbidity from the plasma mixture, if the comparison is made by reflected light with both flasks standing on a white surface. When near the end-point add one drop at a time until an exact match is secured. Read the burette. If the proper end-point has been reached the addition of another drop will make the plasma mixture slightly too yellowish. Always carry the titration to this point. Van Slyke's<sup>1</sup> warning as to the shifting back of the color from yellow to pink when near the end-point applies only to  $\frac{N}{50}$  NaOH containing some carbonate.

Wash the titration flask soon to prevent the neutral red depositing on the glass (ammonia removes the deposit).

*Calculation.*—Determine the c.c. of  $\frac{N}{50}$  HCl neutralized by subtracting the c.c. of  $\frac{N}{50}$  NaOH used from the titration value of 5 c.c. of the acid. Multiply this figure by 22.4. The result is the alkali reserve figure, which agrees closely with the figure obtained by using Van Slyke's apparatus (see Table II).

*Standards for Judging the Results* (Van Slyke).—

Normal resting adults .....	over 50.
Mild acidosis without symptoms .....	40 to 50.
Moderate acidosis which may give symptoms .....	30 to 40.
Severe acidosis with distinct symptoms .....	below 30.

*Titration Value of the  $\frac{N}{50}$  HCl.*—This should be determined (in the manner directed below) each day that estimations are made. A variation of 0.05 c.c. from the correct titration is allowable. If the  $\frac{N}{50}$  NaOH contains a little carbonate, it becomes difficult to secure a satisfactory titration because of the shifting of the color after the addition of each drop of alkali when near the end-point. The following modification has remedied this difficulty. To 20 c.c. of distilled water and 0.3 c.c. of neutral red add 1 c.c. of a weak  $\text{Na}_2\text{HPO}_4$  solution (about 50 mg. per 100 c.c.) and then add  $\frac{N}{50}$  HCl, a small drop at a time, until the mixture matches the clear standard. Now add 5 c.c.  $\frac{N}{50}$  HCl and titrate.

\*Both pipettes and the burette should be tested for accuracy.

*Reagents.*—(1.) Prepare  $\frac{N}{50}$  HCl by exact dilution of a correct  $\frac{N}{19}$  solution. Chloroform may be added to prevent growth of molds. (2.) Prepare carbonate-free  $\frac{N}{50}$  NaOH as follows:—Boil 1 liter of distilled water for about one minute, cork (loosely) and cool. Now add 1.2 c.c. of clear 65 per cent NaOH\* and mix thoroughly. Keep the flask tightly corked. Fill a burette with the solution and cover it with a test tube. Now check the solution by titrating a mixture of exactly 5 c.c.  $\frac{N}{50}$  HCl, 20 c.c. distilled water and 0.3 c.c. neutral red in a 120 c.c. Erlenmeyer flask until it matches the clear permanent standard. Dilute the whole volume of solution with an amount of boiled distilled water, such that the titration will become exactly 5 c.c., and recheck.

The  $\frac{N}{50}$  NaOH should be divided among a number of small nonsol flasks as previously stated, putting 25 to 50 c.c. in a flask. Keep the flasks tightly stoppered. The solution may be used from one flask for one day only, as a rule.

#### RESULTS SECURED USING THE MODIFIED METHOD

It will be seen by referring to Table II that in the case of the 15 plasmas examined the results were very satisfactory. In only two cases is the variation in the alkali reserve figure from that obtained by the standard method greater than 1, the widest variation being 1.5. In order to get such close results it is necessary to read the burette very carefully, within 0.02 c.c. (compare duplicate estimations in Table II).

TABLE II  
MODIFIED TITRATION AND STANDARD METHODS COMPARED

PLASMA	TITRATION METHOD		ALKALI RESERVE FIGURE BY VAN SLYKE'S APPARATUS	VARIATION IN THE RESULTS BY THE TWO METHODS
	$\frac{N}{50}$ HCl NEUTRALIZED C.C.	ALKALI RESERVE FIGURE		
1.	3.23	72.3	71.6	+0.7
2.	2.63	58.9	59.1	-0.2
	2.63	58.9		-0.2
	2.65	59.4		+0.3
3.	2.50	56.0	56.2	-0.2
	2.53	56.7		+0.5
4.	2.45	54.8	54.4	+0.4
5.	2.43	54.4	53.1	+1.3
	2.40	53.8		+0.7
6.	2.25	50.4	50.5	-0.1
	2.23	49.9		-0.6
7.	2.15	48.2	48.1	+0.1
8.	2.18	48.8	47.3	+1.5
	2.15	48.2		+0.9
9.	1.81	40.5	41.4	-0.9
	1.81	40.5		-0.9
10.	1.82	40.8	40.0	+0.8
11.	1.62	36.3	36.7	-0.4
12.	1.44	32.3	32.0	-0.3
13.	1.35	30.2	31.0	-0.8
14.	1.40	31.4	30.8	+0.6
15.	1.25	28.0	28.1	-0.1

\* If this reagent alkali solution is not on hand, it may be prepared as follows: Dissolve 50 gm. pure (99.9 per cent) caustic water, cool and let it settle a couple of days in a tightly corked bottle.

Each plasma was treated as an unknown and the results were not calculated until the estimations by both methods were completed. All the results were secured after becoming expert in the technic.

*Conclusion.*—We feel confident that this new method, as modified above, will prove to be a satisfactory test for acidosis even in the hands of the average practitioner.

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Haskins, Howard D.: Northwest Med., 1918, xvii, 35.  
<sup>3</sup>Haskins, Howard D.: Jour. Lab. and Clin. Med., 1919, iv, 363.

## A COMPARISON OF THREE METHODS OF EXAMINING SPUTA FOR B. TUBERCULOSIS

BY LLOYD R. JONES,\* GREENVILLE, S. C.

TEXTBOOKS ordinarily refer to two methods of preparing sputum specimens for examination, namely, a smear made directly from the expectorated material choosing the gray, purulent particles and smears made from the sediment of the specimen after some method of concentration has been used. For the latter procedure the use of antiformin is often advocated. Goeckel<sup>1</sup> has recently advised the use of Rice's Bromine and Alkali reagent as a substitute for antiformin. Antiformin, first used by Uhlenhuth<sup>2</sup> in bacteriologic studies and now in extensive use, is a strong solution of sodium hydroxide and sodium hypochlorite, and is a very potent oxidizing agent, dissolving many kinds of organic matter and most microorganisms. The tubercle bacterium, however, probably owing to its waxy-like capsule, is quite resistant to this oxidation process and remains intact with normal staining characteristics.

At this Laboratory where from 50 to 160 specimens of sputum are examined daily we have subjected all of them to 15 pounds of steam pressure for 15 minutes in an autoclave. This process, of course, coagulates all mucus and serous material and renders innocuous tubercle bacilli if present, as well as all other microorganisms. The coagulum with its entangled bacteria is of suitable consistency for preparing the smear.

In order to compare the value of the various methods in the routine examination of sputa, the writer selected a ward with seventeen patients from whom the expectorations between the hours of 12 midnight and 6 A.M. were collected in the ordinary type of sputum container and brought to the laboratory for examination. As the amount of sputum would not permit all three methods of examination to be made from a single specimen, the plan of using one each day was used.

Direct smears were made by selecting from the tenacious material the purulent particles for examination.

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With the antiformin method the reagent of 30 per cent strength was added to the sputum in centrifuge tubes (the volume of each being equal) and allowed to stand from 10 to 12 hours in which time digestion of the tenacious mass was completed. The tubes were then centrifuged at a high rate of speed for 15 minutes and smears made from the sediment. With the autoclave method, thick smears were prepared from the coagulum.

All prepared smears were fixed to the slide by heat, stained with steaming carbol-fuchsin for from 5 to 10 minutes, decolorized with acid alcohol (2.5 per cent hydrochloric acid in 95 per cent ethyl alcohol) and counterstained with a saturated alcoholic solution of picric acid. This particular method of counterstaining gives very satisfactory results in our hands in that it gives to the preparation a soft yellow color that is easily penetrated by light and makes possible the examination of thick smears in that the microscope may be focused up and down through the mass, thereby improving the chances of finding the acid-fast organisms if they are not abundant, since relatively larger amounts of material are examined.

In recording the results of microscopic examination we have used Gaffky's scheme<sup>3</sup> tabulating the average number of bacteria per field in accordance with the following (See Table, page 42):

(GAFFKY'S SCHEME AS MODIFIED BY L. BROWN)

1. Only 1 to 4 in a whole preparation.
2. Only 1 bacillus on an average in many fields.
3. Only one bacillus on an average in each field.
4. 2 to 3 bacilli on an average to each field.
5. 4 to 6 bacilli on an average to each field.
6. 7 to 12 bacilli on an average to each field.
7. 13 to 25 bacilli on an average to each field.
8. About 50 bacilli on an average to each field.
9. 100 or more bacilli on an average to each field.
10. Enormous numbers on an average to each field

From the foregoing it is noted that the concentration of bacilli in tuberculous sputum is slightly more after treatment with the antiformin than with the autoclave method. Neither to any extent surpass the direct smear examination.

Among the direct smear examinations, negative sputa were found three times on two patients. With the antiformin method negative sputa were found eight times on five patients and with the autoclave method only two negatives were found in the whole series and those on one patient. That is to say of a total of 153 examinations by the direct method three were negative, of a total of 170 by the antiformin method eight were negative, and of 170 examinations by the autoclave method only two were negative.

The autoclave method kills all the tubercle bacilli and the material is subsequently easy to handle and quite safe so far as the danger of disseminating the infection is concerned and where large numbers of specimens are to be examined the method is most convenient.

The writer is greatly indebted to Assistant Bacteriologist Helen E. Kellogg and other assistants in making the examinations and in compilation of the above data.

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<sup>3</sup>Simon: *Clinical Diagnosis*, p. 288.

## THE ESTIMATION OF CHLORIDES IN WHOLE BLOOD\*

BY JOHN B. RIEGER, S.M., M.D., DETROIT, MICH.

AS a method of precision, the determination of chlorides in whole blood, according to Van Slyke and Austin,<sup>1</sup> leaves little to be desired. The application of the Volhard titration to the protein-free filtrate obtained by the Folin system of blood-analysis,<sup>2</sup> is, however, entirely feasible and will be preferred by some. Rappleye<sup>3</sup> has successfully applied the principle to plasma, and his technique with slight changes may also be used on the filtrate obtained by tungstic acid precipitation, provided the reagents be free of chloride.

## PURIFICATION OF SODIUM TUNGSTATE

A ten per cent solution of sodium tungstate is acidified with an equal volume of concentrated nitric acid, the lemon-yellow precipitate filtered off, and to the filtrate which has remained clear following the addition of a few more drops of nitric acid, is added a few drops of silver nitrate test solution. Any turbidity that may be seen by transmitted light indicates an appreciable chloride content.

To purify, the tungstate solution is poured into an equal volume of fifty per cent sulphuric acid contained in a tall cylinder, the precipitate allowed to settle and the supernatant fluid syphoned off or sucked off through a Büchner funnel. The precipitated acid is washed by decantation until the test for chlorides is no longer given; it is then dissolved in the requisite amount of 40 per cent sodium hydroxide (seven c.c. for each ten grams of sodium tungstate taken) and the reaction adjusted with dilute sulphuric acid, until neutral to litmus. Enough water is then added to make a solution of 1.15 sp.gr. This is filtered and is then ready for use.

## SOLUTIONS REQUIRED

## 1. Standard silver solution.

Silver nitrate crystals,	7.2653 g.
Nitric acid, sp.gr., 1.42,	250 c.c.
Sat. sol. iron-ammonium alum	50 c.c.
Distilled water ad	1000 c.c.

2. Ammonium sulphocyanate solution, 0.75 g. in 1000 c.c., to be adjusted by titration so that 25 c.c. equals five c.c. of the silver solution.

3. Solution of sodium oxalate, recrystallized, 1 g. to 100 c.c. One c.c. of the solution is put in an ounce salt-mouth bottle, and evaporated to dryness. The oxalate forms a thin film over the bottom and is sufficient for 10-15 c.c. of blood.

4. Chloride-free, neutral 10 per cent sodium-tungstate solution, sp.gr. 1.15.

5. Sulphuric acid, 2-3 normal.



## THE DETERMINATION

One volume of sodium tungstate solution is placed in a suitable flask, one volume of oxalated blood is added, followed by one volume of 2.3 normal sulphuric acid. The flask is well agitated and allowed to stand for an hour or longer, preferably in an ice box, for protection of organic constituents. The blood is then diluted to ten volumes, the flask agitated, and the contents filtered. The filtrate should be water-white, and give no precipitate with an equal volume of nitric acid (absence of tungstate). The presence of tungstate greatly obscures the end-point in the succeeding titration. Twenty c.c. of the filtrate, representing 2 c.c. whole blood, is placed in a 50 c.c., volumetric flask, 10 c.c. distilled water added and 10 c.c. of the standard silver solution. Dilute to the mark, shake vigorously to coagulate the silver chloride, centrifuge or filter. Twenty-five c.c. of the filtrate is then titrated with ammonium sulphocyanate to the appearance of the first brown tinge. This is quite sharp. The number of c.c. of the cyanate solution is subtracted from 25 and the difference multiplied by 50 to obtain the number of milligrams of sodium chloride per 100 c.c. of whole blood.

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## A NOTE ON THE STABILITY OF DRAWN BLOOD\*

BY H. L. WHITE, PH.D., AND THOMAS WATSON, M.A., LOS ANGELES, CAL.

THE question is frequently asked: What changes take place in samples of drawn blood, upon standing for several hours, that may affect the value of the results of chemical analysis for clinical purposes? As frequently happens, samples of blood obtained during early afternoon office hours may not reach the analyst before the following morning. In sparsely settled sections, samples of blood must be sent to the analyst by mail or express. In either case there is a delay of from sixteen to twenty-four hours before the analysis is begun. Another factor involved is that of temperature. While some samples may be placed in a refrigerator, others must be kept at room temperature for several hours with the possibility of some changes in composition taking place.

A few references have been noted in the literature upon the keeping qualities of blood, but they are inconclusive and leave the general impression that changes take place rapidly. The analyst is advised by the authors of one manual<sup>1</sup> to estimate sugar and creatinine at once "because these two substances most quickly deteriorate."

In order to be able to answer the question as to the changes taking place in blood, we obtained samples of ox blood and of human blood which were analyzed

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<sup>1</sup>Gradwohl and Blaivas: *Blood and Urine Chemistry*, 1917.

TABLE I  
NONPROTEIN AND UREA NITROGEN  
Mgs. per 100 c.c. Blood

LAB. NO.	KIND OF BLOOD	AGE OF SAMPLE (HOURS)	TEMPERATURE	NONPROTEIN NITROGEN	UREA NITROGEN
20	Ox Blood	1	Room	23.0	11.0
20 (a)		24	Room	21.0	8.0
20 (b)		24	Refrigerator	20.0	10.0
18		1	Room	43	16
18 (a)		25	Room	42	—
18 (b)	Human Blood	25	Refrigerator	49	—
18 (c)		168	Refrigerator	—	15
3		1	Room	26	16
3 (a)		18	Room	27	14

as soon as possible after being drawn. The remainder of the sample was divided into two parts, one of which was placed in a refrigerator, while the second was allowed to remain at laboratory temperature. These conditions are indicated in Tables I and II as "Refrigerator" and "Room." The samples were collected in clean, but not sterile containers, with sufficient potassium oxalate added to prevent coagulation. The analyses were made by the methods of Folin and Wu. The results are shown in Tables I and II.

TABLE II  
CREATININE, URIC ACID AND SUGAR  
Mgs. per 100 c.c. Blood

LAB. NO.	KIND OF BLOOD	AGE OF SAMPLE (HOURS)	TEMPERATURE	CREATININE	URIC ACID	SUGAR
A	Rabbit Blood	5 (minutes)	Room	1.3	—	320
AI		2	Room	1.4	—	310
15	Ox Blood	1	Room	1.1	2.3	243
15 (a)		20	Room	1.1	2.2	204
15 (b)		20	Refrigerator	1.1	2.1	208
15 (c)		44	Room	—	—	142
15 (d)		44	Refrigerator	—	—	168
15 (e)		144	Room	—	1.4	none
15 (f)	Human Blood	144	Refrigerator	—	3.6	46
9		3	Room	1.0	—	120
9 (a)		27	Room	1.5	—	110
3	Human Blood	1	Room	1.9		
3 (a)		18	Room	1.8		

The results obtained in this preliminary investigation lead us to believe that the changes taking place in blood, as shown by ordinary chemical estimations, are slight in character, and that a sample of blood may be kept in a clean container for about twenty hours at room temperature without undergoing any marked decomposition. This work is being continued.

We wish to acknowledge our indebtedness to Mr. Charles Drabkin, a student in this College, for his very efficient aid in the analytical work.

## BACTERIAL VACCINES—CHLORETONE SOLUTION AS A VEHICLE FOR THEIR ADMINISTRATION\*

By R. G. OWEN, M.D.; F. A. MARTIN, M.D.; AND W. L. BROSIUS, M.D.,  
DETROIT, MICH.

IN A recent article in this JOURNAL, Aldrich and Ward<sup>1</sup> discussed the use of chloretone (Trichlorotertiary Butyl Alcohol) and mentioned its anesthetic and bactericidal powers, stating that it would not only prevent the growth of moulds, but would kill all but the most resistant spore bearing germs. It also exerts marked local anesthetic effect with some hypnotic and general anesthetic action. In addition relatively large doses can be taken with impunity.

For several years we have been using a saturated solution of chloretone to wash our bacterial suspension off the agar slants and to dilute the bacterial mass to the proper number. After the desired dilution has been made, the vaccine is heated in a water-bath at 55-60° C. for one hour and the usual control cultures made. The chloretone will, of itself, kill all the organisms present without heat, but may require several days to do so.

There is no clumping of the organisms as seen when Tricresol or carbolic acid is added and the resulting vaccine is perfectly homogenous, sterile, and remains so. Moreover, its anesthetic properties make the use of such a vaccine most acceptable to the patient.

It is not necessary to sterilize the chloretone solution before use. We simply make up a saturated solution in cold distilled water, keep it for several days and test to insure sterility. Once sterile it will remain so indefinitely.

### Errata

In the editorial entitled "Recent Researches on the Capillary Circulation" in the September number of this Journal (Vol. V, No. 12, p. 803), the word *methane* is used instead of *urethane* (on pp. 805 and 806). This error has arisen because of the impossibility of submitting proof to the writer of the article who was overseas.

In the July issue of the Journal the editorial entitled "Recent Work on Vitamines" should be signed J. J. R. Mc.—not F. C. F.

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<sup>1</sup>Jour. Lab. and Clin. Med., 1920, v, 583.

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## EDITORIALS

### *Isolation of the Specific Hormone of the Posterior Portion of the Pituitary Gland*

FOR a quarter of a century a peculiar and fascinating interest has been attached to the study of the ductless glands. The literature on this subject has grown to enormous proportions, and in many phases of this obscure field, has far outrun the real progress of our actual knowledge of the subject. The gradually increasing clinical utilization of preparations obtained from the endocrine glands has served to still further stimulate investigation along these lines.

Some twenty years ago Abel<sup>1</sup> isolated the active principle of the adrenal glands and named it epinephrine. And within the last year this investigator and his coworkers have again made most valuable contributions to our knowledge of internal secretions. On this occasion, however, the posterior, or infundibular, portion of the pituitary gland has been studied. Notwithstanding the long period of time which has elapsed since Schäfer and Oliver<sup>2</sup> discovered that crude extracts of the pituitary gland would raise blood pressure, still the true active principle of the posterior portion of this gland has not been isolated and identified as a pure chemical substance.

Abel<sup>3</sup> and his collaborators have recently published three papers on this subject, of which the last is of the most immediate interest.

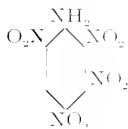
In 1912 Guggenheim<sup>4</sup> discovered that pituitary extracts caused contraction of the uterus in the rat, but that histamine, ( $\beta$ -iminazolyethylamine, "ergamine") caused, on the contrary, relaxation and reduction of tonus in this organ. Two other experimenters<sup>5</sup> have recently found that in the dog histamine is a very intense bronchoconstrictor, but that those pituitary extracts which contain not more than traces of histamine are without action on the bronchioles of this animal. On the contrary, both pituitary extracts and histamine produce very marked uterine contractions in the dog. But commercial preparations of the pituitary gland frequently contain small, variable amounts of some substance acting in a manner exactly similar to histamine. These preparations produce bronchoconstriction in proportion to the amount of this substance present. And these authors also suggest that in all probability the true active principle of the posterior portion of the pituitary gland is a simple body of the sympatho-mimetic amine type which most likely acts by stimulation of the endings, or myoneural junctions, of certain nervous elements, which very likely belong to the thoraco-lumbar division of the autonomic nervous system. Dudley<sup>6</sup> has also verified Guggenheim's earlier observation that alkali will destroy both the pressor and the muscle stimulating activities of a pituitary extract in a concentration which leaves the action of histamine on the blood-pressure unaltered. And Dudley has also shown that butyl alcohol will readily extract the uterine stimulating substance from acid solution, while under the same circumstances histamine is only very slowly extracted. And it may be further noted that the active principle of the pituitary gland is insoluble, while histamine is soluble in boiling chloroform, and digestion with trypsin rapidly destroys the active pituitary principle but leaves histamine unchanged. Dudley's observations have been confirmed and extended by Abel and Nagayama.<sup>3</sup> And further these authors have shown both chemically and pharmacologically that commercial pituitary extracts contain small amounts of histamine. This seems to be a result of the methods used in the preparation of these extracts, for they find that infundibular extracts that have been prepared with care from fresh glands, without boiling or long exposure to acids, contain only small amounts of free histamine, but commercial extracts may contain a much higher proportion of this substance. It seems that histamine may thus be derived from the decomposition of some, as yet unknown, constituent of the posterior lobe. Of still further interest, however, is the finding of Abel and Nagayama that infundibular extracts contain not one, but two, depressor substances. And in addition they have isolated in the form of slightly impure salts (phosphate, pierate and a tetranitroaniline compound) a substance that appears without doubt to be the true active principle, or hormone, of the posterior portion of the pituitary gland. This latter substance is of great scientific and practical interest, for it seems exceedingly probable that the body will be thoroughly purified and identified in the near future. It is very likely that the substance can then be manufactured synthetically at a very much smaller cost than the ordinary commercial pituitary extracts are now prepared. Abel and Nagayama have further shown that histamine and also the second depressor substance discovered by them are not specific for the pituitary gland, but can

be readily extracted from a great variety of other tissues. This is especially emphasized in another paper by Nagayama<sup>7</sup> in which he shows that both histamine and a histamine-like depressor substance, which is apparently identical with the second depressor body obtained from infundibular extracts, can readily be isolated from the ordinary albumoses and so-called peptones of commerce. The authors also find that tryptic digestion of infundibular extracts change their blood pressure raising action into a depressor one, while simultaneously there is a progressive increase in the bronchoconstrictor action in decerebrate cats. This same change can also be brought about by mild hydrolysis with acids. Regarding the chemical properties of the two depressor bodies in pituitary extracts, it seems entirely proved that one is histamine. This is soluble in chloroform and gives the Pauly reaction but not the biuret reaction, while the second histamine-like depressor substance is *insoluble in chloroform*, but is soluble in 95 per cent ethyl alcohol. It likewise gives a positive Pauly but negative biuret reaction. The depressor and oxytotic actions produced by infundibular extracts in which the *pressor* principle has been destroyed, is estimated to be caused to the extent of about 20 per cent by histamine and 80 per cent by the histamine-like substance. This latter substance has not yet been isolated in an absolutely pure form, but it seems probable that this will be effected in due time.

Regarding the pressor principle, which presumably represents the true specific hormone of the posterior portion of the pituitary gland it may be said that this substance gives both the Pauly and the biuret reactions. The phosphate salt is especially adapted to chemical tests. Aqueous solutions of either the picrate or the phosphate show an acid reaction toward litmus, which indicates that the pressor substance is a very weak base. Neither salt has yet been obtained in an indubitably crystalline form, and hence the authors cannot be sure they are dealing with a *single* chemical individual. When the substance, in the form of its salts, is hydrolyzed with acids, the biuret reaction is lost entirely, but the Pauly reaction remains unchanged. Treatment of the substance with acids apparently gives rise to a certain amount of histamine and also of the histamine-like depressor substance.

Pharmacologically this substance raises blood-pressure in a decided manner, while its oxytotic action is many times more powerful than that of histamine. In fact the action of the substance on the uterus of the guinea-pig was so extraordinary that the authors have preferred to withhold the tracings for corroboration in experiments to be carried out later. It is of great significance that treatment of this substance with acids produces a certain amount of histamine and a larger amount of the histamine-like depressor substance, while at the same time all pressor action is lost. Other physiologic properties which these substances may possess, such as their action on diuresis, etc., have not yet been studied. But the vascular constricting substance evidently loses its pressor action in an animal after a few intravenous injections, given at short intervals of time. This closely resembles the blood-pressure action of ordinary commercial pituitary extracts.

A feature of this work which promises to yield valuable results in the future is the introduction of the use of tetranitroaniline,



in the isolation and purification of the pressor substance. The pressor substance forms a condensation product with the tetranitroaniline in the same manner as amino acids and peptids combine with  $\beta$ - and  $\gamma$ -trinitro-toluene as shown by Barger and Tutin.<sup>8</sup> The histamine is first removed from the crude pituitary extracts by chloroform. Certain features, however, of the special work of isolating the pressor substance the authors have preferred to subject to further study, as they believe that their present methods can be improved upon in various details.

Regarding the exact chemical nature of the pressor substances, the authors suggest that it may be a compound in the nature of a slightly basic, unstable peptamine, or, of the nature of a relatively simple but unstable, peptid. In either case they assume that an imidazol ring is present.

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—D. E. J.

### American Relief Work In Vienna

IN THE May first number of the *Weiner Medizinische Wochenschrift* there appears as the leading article an appreciation of the work done by the American Food Relief Commission in Austria. The writer, Dr. Clemens Pirquet, Professor of Pediatrics in the University of Vienna, does not hesitate to praise unqualifiedly both the motives under which the work was begun, and the results that have been accomplished.

According to his report, the representatives of our government first undertook to feed the starving children of Austria in May, 1919. The work was officially inaugurated on June 2, although the feeding of children had been commenced as early as May 26. On June 2, there were six food stations supplying nourishment for 6,000 children. One month later the daily rationing in Vienna totaled 75,000 meals; on July 8, 95,000, and on the 16th, 106,478. Within a month and a half the objective set by the Relief Commission had been over-reached. The distribution of food through Austria was proportioned, 50 per cent to Vienna, 16 per cent to the rest of Lower Austria, 5 per cent for Upper Austria, 2.5 per cent to Salzburg, 5 per cent to Tyrol, 2.5 per cent to Vorarlberg, 15 per cent to Steiermark, and 4 per cent for Kärnten. In all, 200,000 children were cared for daily. By autumn the maximum number had been increased to 218,000.

In Vienna stations were established in former palaces and castles, in buildings previously used as army kitchens, in barracks, kindergartens, day nurseries, hospitals, convalescent homes, orphan asylums, etc. The largest station ultimately fed 13,000 children daily. Three thousand were fed daily in recreation or convalescent camps, 10,000 in institutions, 16,000 in semi-institutions, kindergartens, etc., and 76,000 in the large general kitchens. The greater the number fed from the general kitchens, the greater was the assurance that only those who were in greatest need of food and who were least able to obtain it were being fed. At all times the selection of children for feeding has been based upon their physical state and needs. Hunger and want among the older members of the family could play no role in selection, neither could any attempt be made to relieve these others. The aim of the work was to feed the children and the lines were drawn fast. Nursing mothers were fed in behalf of their infants.

Pressed with work as they have been, the distributors have not lost sight of the human side of life. On Christmas 14,000 kgm. of flour, 1700 kgm. of sugar, 5000 cans of milk and 750 kgm. of lard, etc., were converted into over 100,000 Christmas cakes. These were distributed at nearly 200 points in the city on Christmas day.

In November a survey was made, with the help of physicians, of the schools of Vienna, to discover those children in need of aid who had not as yet been cared for by the commission. One hundred eighty-six thousand, six hundred seventeen children were examined, of whom 96,789 were severely undernourished, 63,402 undernourished, 19,694 mildly so, and 6,732 not undernourished. On December 15, all children who had been receiving food for more than eight weeks, many of them for five and six months, were discontinued, to make place for other needy ones. A similar survey and redistribution was made two months later. On a physician's recommendation any child would be replaced in the relief list, or could be continued a longer time. According to the November survey, 79,604 of the 96,789 very undernourished children received help from the commission, 42,264 of the 63,402 undernourished, and 11,952 of the 19,647 mildly undernourished. Twenty-three thousand eighty-six were not fed.

Some idea of the ill-fed condition of Viennese children is gained when we consider that in a survey of 186,617, in twenty-one districts of the city, the highest per cent of seriously undernourished was 63 in district 13, the lowest, 31.1 per cent in district three, while the median for all districts was 48 per cent.

"German Austria, dying under the difficult burden of the years of destruction, was in the list of those countries which were to receive the singular aid, truly singular in that it happened, un hoped for after this rage of hate, that even before the conclusion of peace the reconciling hand assisting and healing the wounds should be offered to the enemy."

This appreciation by Dr. Pirquet written to the physicians of Austria, coming as it does at a time when official America apparently shamelessly ignores its share of the burdens of humanity, and when the rest of us consider ourselves too busy or are too indifferent to protest, serves to remind us that we are accomplishing something, if not all, of which we are capable.

—H. T. I'.



## *Roentgenology and the Internist*

IN an all too brief discussion of the value of the x-ray to the internist, Dunn has made a real addition to medical perspective, or perhaps it were better to say he has written something that should deepen clinical perspective. He calls attention to the fact that the roentgen ray reveals only variations in density; and that these variations are the results of pathologic processes, that the roentgen methods have to do with the results of processes, not with the processes themselves. Fluoroscopic methods on the other hand in which motions are visualized give more information of physiologic trend. So, radiology, just in so far as it is a morphologic method, is handicapped, for clinical medicine is an affair of physiology, that is to say, of physics and chemistry, not one of morphology.

This does not mean, however, that radiology is not valuable for the internist, provided that it is not abused. The reverse is true. But its limitation should be appreciated and observed by radiologist, clinician and patient. Radiology except as it may make an absolute diagnosis is an adjunct, a part of a clinical examination, just as is urinalysis, serologic findings and many other procedures. The term *x-ray diagnosis* is widely used. These various proceedings result in *findings* not in *diagnoses*, as a rule. They give additional facts, which, placed together with other facts, make it possible to arrive at a diagnosis. The finding of an enlarged sella turcica, or of an abnormal shadow in a lung, is an important fact, but it does not necessarily reveal the morbid physiology at work, and it tells nothing of the correlated processes elsewhere in the body. The roentgen ray reveals the results of processes, but not how such processes came into being. With respect to the immediate value of the x-ray in making medical diagnoses, Dunn has this to say: The diagnosticians "batting average" varies in direct ratio to the number and efficiency of the methods he employs in the study of his cases. One of these methods is the radiologic. All should be used, and as Dunn infers, radiology is just as useful and should be used with almost the same frequency as is urinalysis. It is a misfortune that in too many instances the roentgen ray is used merely when it is expected to be a positive factor in arriving at a diagnosis. The value of negative x-ray findings seems to be much neglected. When a patient complaining of weakness, of rapid heart action, and of loss in weight, and without goitre, gives a positive von Pirquet and slight febrile manifestations, it is obviously of value to know that there are no abnormal shadows in the lungs. The differential diagnosis of latent pulmonary tuberculosis, from myocarditis, endocarditis, Graves' disease, latent syphilis and neurasthenia demands a resort to all diagnostic methods of proved worth. It is just in this most difficult class of cases that disturbances in lung and mediastinal densities are most often of crucial importance in determining the conditions at work and it is the experience gained by the routine use of the roentgen method that stands one in stead when the problems grow difficult.

In estimating the position that the x-ray takes in his own work, Dunn says that it gives him decisive information in about one case in twenty, exclusive of examination of the teeth. He estimates that the routine use of the method would add from five to ten per cent to a good diagnostician's efficiency.

Finally Dunn insists that radiology should be practised by specialists, and that their duty in diagnosis shall be confined to collecting data—diagnostic suggestions—to be added to the other clinical findings.

## REFERENCE

Dunn: Jour. of Roentgenology, Sept., 1919.

—P. G. W.

### *Are There Two Diseases Included Under the Present Diagnosis of Smallpox?*

STUDENTS of the history of infectious diseases have long suspected that under the diagnosis of smallpox we include two closely related but different diseases—the Asiatic and the African varieties. That smallpox existed in India in the third century B. C. is shown by some of the medical writings of that period. There were special prayers which were repeated by the Brahmin priests while performing the operation of inoculation for this disease. That smallpox existed in Egypt even at a remote period, as early as 1200 B. C., has been shown by Ruffer and Ferguson from eruptions found on the skin of a mummy belonging to that period. So far as epidemiologists are able to trace the Asiatic variety it spread from India into China and then gradually westward into Asia Minor. We would know but little about smallpox during the ninth and tenth centuries were it not for the writings of Arabian physicians. Apparently, it took centuries for Asiatic smallpox to travel from eastern India and China to Turkey in Europe. Whether the smallpox that finally spread over Europe came from Egypt or from Asia is a matter of speculation. Moreover, Egyptian smallpox may have been identical with the Asiatic form of the disease, and still there might have been an Ethiopian variety which was not known to the white man until comparatively recent years. During the entire history of smallpox severe and mild epidemics have occurred not only in different localities, but in the same locality at different times. Under the name "alastrim" or "Kafir milkpox," Castellani and Chalmers<sup>1</sup> recognize the African or mild form of smallpox. Concerning its causation and nature, these authors make the following statement:

"The causation would appear to be the same as ordinary smallpox, but it is generally agreed that Jenner's vaccination is protective, and Guarnieri bodies have been found, and the classical reaction in the inoculated cornea of the rabbit has been produced; after sixty hours the Guarnieri bodies have been recovered from the cornea, but it would appear to be due to an attenuated virus. The question which has been much debated is whether it is smallpox, chickenpox, or a new disease halfway between the two."

Schamberg<sup>2</sup> states that "alastrim" apparently first appeared in this country in Florida in 1896. He rejects the idea that it was imported from Cuba in 1898. He states that the period of incubation is longer than that of normal smallpox, being from fourteen to eighteen days. The initial stage is so mild that evidences of illness are not recognized even by the patient himself. The same author continues by saying that in this country hundreds of thousands of cases have developed and the disease has continued to maintain its mild character and is not

reverting to the normal virulence of classical smallpox. "Two phenomena of importance stand out in connection with this type of smallpox: (1) The disease occurs almost exclusively among the unvaccinated; a single vaccination, no matter how remotely performed, is protective in the vast majority of instances. Negroes vaccinated during the Civil War, living in the same household with persons suffering with this disease, were completely protected over forty years later. This is a common circumstance. In Carbondale, Pa., in 1912, ninety-six out of ninety-seven patients who took this type of smallpox were unvaccinated, and there was some doubt about the vaccinal condition of the remaining patient. The patients who are immune against this type of smallpox are not necessarily immune against smallpox of normal virulence. (2) As has been stated, the symptomatology of this affection is mild and the disease is accompanied by a very low mortality, varying from one-half to two per cent. In the epidemic referred to in Carbondale, Pa., there were no deaths. At the same time, smallpox of European importation was prevalent in Pittsburgh, Pa.; there were one hundred fourteen cases with thirty-one deaths, a mortality of twenty-seven per cent. We note, therefore, that the disease is characterized by a mild symptomatology and a mild infectivity. As to the cause of this deviation from classic smallpox no definite statement can be made. Doubtless, as a result of some unknown cause, the parasite which produces this disease was attenuated in virulence; the phenomenon is what biologists would call a 'sport.' Whether or not this type of smallpox will continue to retain its present mild form no one can predict. It is possible that at some future time it may revert to the usual virulence of the disease."

Recently Allingham<sup>3</sup> has reported three cases of modified smallpox, with the following remarks:

"Reviewing these cases, which occurred without any history of exposure to smallpox, the diagnosis of chickenpox on the first rash was, in my opinion, reasonable, and it was only after the first patient developed a secondary rash that I decided to isolate the subsequent cases at an early date. It might be wise to insist on isolation of chickenpox for the first ten days."

#### REFERENCES

<sup>1</sup>Manual of Tropical Medicine, Wm. Wood & Co., 1913.

<sup>2</sup>Diseases of the Skin and the Eruptive Fevers, W. B. Saunders Co., Philadelphia, 1917.

<sup>3</sup>The Lancet, London, May 29, 1920.

I. C. I.

### *The Life-History of the First Case of Myxedema Treated by Thyroid Extract*

MURRAY,<sup>1</sup> after studying the effects produced by the entire removal of the thyroid gland by Horsley in animals, determined in 1891 to treat myxedema with an extract of these glands. The first patient upon whom this experiment was made was at that time a woman of forty-six years of age. Her condition was as follows:

"She complains of languor, a disinclination to see strangers, and great sensitiveness to cold. The temperature is subnormal, and varies between 95.6° and

<sup>1</sup>British Medical Journal, March 13, 1920.

97.2 in the mouth. The pulse varies between 60 and 70. The face is blank and expressionless and the features are notably thickened. This change is well seen in the alae nasi and lips. The subcutaneous connective tissue of the eyelids is so swollen that she finds it difficult to look upwards. There is also considerable swelling beneath the eyes and of the cheeks. The hands and feet are both enlarged; the former have that peculiar shape which has been described as spade-like. The skin is very dry, there is no perspiration, and the superficial layers of the epidermis are continually being shed as a fine white powder. The hair is very fine in texture, and a considerable quantity of it has been lost. She is slow in answering questions; all her actions are slow and are performed with difficulty. The speech is remarkably slow and drawling and the memory is bad. No thyroid gland can be felt in the neck. The urine contains no albumin or sugar."

Murray prepared and administered a glycerin extract of the thyroid gland of a sheep, and three months later the condition of the woman is described as follows:

"The swelling has gradually diminished, and has practically disappeared from the backs of the hands, the skin over them being now loose and freely movable. The lips are much smaller. The swelling of the upper eyelids has diminished so much that she can look upwards quite easily. The swelling beneath the eyes and of the cheeks has also much diminished. The face consequently, as a whole, has greatly improved in appearance and has much more expression, as many of the natural wrinkles, especially about the forehead, have returned. The speech has become more rapid and fluent, the drawl being scarcely noticeable at the present time. She answers questions much more readily, the mind has become more active, and the memory has improved. She is more active in all her movements, and finds that it requires much less effort than formerly to do her housework. She now walks about the streets without any hesitation without a companion. She has menstruated normally during the last six weeks at the regular interval. For the last four weeks the skin has been much less dry and she perspires when walking. The hair remains as before. She is no longer so sensitive to cold. Unfortunately, owing to circumstances a daily record of temperature has not been kept, but out of four observations that have been made lately, about 11 a.m., three times the temperature has been 98.2° and once 97.4."

For some time the thyroid extract was administered at intervals of two weeks subcutaneously twenty-five minims at a time, although during the first three or four weeks this amount was given twice a week. After Mackenzie had shown in 1892 that administration by mouth accomplishes the same purpose, this woman took ten minims by the mouth six nights out of each week. In 1918 when it became difficult to obtain glycerin extract she was fed on thyroid tablets. This woman continued in most excellent health until early in 1919 when she developed edema of the lower extremities and died in May of that year at the age of seventy-four from cardiac failure. During the twenty-eight years Murray computes that this woman consumed over nine pints of liquid thyroid extract or its equivalent prepared from the thyroid glands of more than 870 sheep.

—I. C. I.

### *The Dietetic Treatment of Diabetes Mellitus*

WILLIAMSON<sup>1</sup> advises complete rest for a period of seven days. The patient need not remain in bed, but he should keep the prone position during this time. The only food permitted during the seven days is a mixture of casein, cream and water, given every two hours from 8 A.M. to 10 P.M. If casein is not obtainable in good quality the following prescription may be employed in its stead: Three eggs are beaten up with three ounces of cream in a little water. More water is added gradually until the mixture measures four pints. Of this mixture the patient takes half a pint every two hours from 8 A.M. to 10 P.M. In addition he may take coffee or tea at 8 A.M. and 4 P.M. and a half pint of warm beef tea at noon, 6 P.M. and 10 P.M. No bread, no meat, and no other foods are permitted during this period. According to Williamson glycosuria promptly disappears in suitable cases under this diet, and at the end of the seven days a solid diet containing a calculated amount of carbohydrate is given. If sugar returns, as it may after a long period, the liquid diet is again instituted for a period of seven days. In some instances it has been found more convenient to place the patient upon the liquid diet two days out of every week, while during the remaining five a small quantity of bread or other carbohydrate-containing food, as well as meat, may be permitted. Williamson admits that this form of treatment fails in some cases and that it is without avail when there is marked acidosis. Most patients take the egg and cream diet for seven days quite satisfactorily. Some state that they feel remarkably well on it; others feel rather weak; only rarely do the patients complain of slight sickness, and in such cases, if casein has been employed, eggs should be substituted.

—H. C. F.

### *Influenza Among the Lapps*

MACKLIN<sup>2</sup> was with the British Forces in northern Russia during the influenza epidemic of 1918 and was sent to investigate this disease among the Laplanders. Unfortunately, he is not able to give us any data of scientific value, however interesting his report may be. He states that these people dealt with their sick in a very unsympathetic way. A single-room hut was selected in each village and into this each patient as he came down with the disease was unceremoniously pushed and no one was permitted to return to his own hut until completely recovered. This might be properly designated as a detention hospital. Whilst in the hospital patients received practically no attention and no healthy person ever entered the hut occupied by the sick. Occasionally a bowl of water or reindeer milk was hastily passed in at the door or a huge chunk of reindeer meat thrown in uncooked and uncarved. The patients, consisting of both sexes and all ages, soiled with their own excretions, were crowded in the hut, and often the dead mingled with the living. Fortunately, constipation seemed a constant factor and in many cases there was no movement of the bowels for seven or more days.

<sup>1</sup>British Medical Journal, March 20, 1920.

<sup>2</sup>British Medical Journal, April 3, 1920.

The English Medical Officer was able to supply at least some of these people with more appetizing food, but this was about all that could be done. Bedbugs swarmed over the sick, dying, and dead in these huts, but Macklin saw no other body parasite among the Laplanders, either well or sick. He guesses that about fifty per cent of all the cases of influenza terminated fatally.

—I<sup>r</sup>. C. I<sup>r</sup>.

### *Raise In Subscription Price*

Beginning with the October issue THE JOURNAL OF LABORATORY AND CLINICAL MEDICINE will be \$6.00 per year, and \$6.40 under foreign postage. In view of the increased cost of both labor and material in the production of printed matter, the publishers are compelled to increase the subscription price and feel that this course is preferable to lowering the quality of the JOURNAL.

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## ORIGINAL ARTICLES

### HEMOSTATIC AGENTS AND THE SPONTANEOUS CHANGES IN COAGULATION TIME FOLLOWING HEMORRHAGE\*

By P. J. HANZLIK, M.D., CLEVELAND, OHIO

IN a recent number of this JOURNAL, (February, 1920, No. 5, p. 574) H. C. Hamilton, of Parke, Davis & Co., describes what he considers a superior method for testing hemostatic products, namely, observations on coagulation time of blood before and after intravenous injection. As he points out, the principle of this method has been tried by us in our work on the general subject of the comparative hemostatic efficiency of thromboplastic agents. The experiments that we had tried according to this principle, and which we described briefly in that paper, led us to the conclusion that this principle is not usable.

Hamilton considers that our results were unusual and exceptional, and thinks that they can only be explained by large hemorrhage, which he assumed to have occurred. In this he was mistaken. The quantities of blood that were withdrawn were about 4 c.c. at a time.

In view of this misunderstanding, it is well to give further details that were omitted from our original paper, because we considered them sufficiently implied in the data given. Dogs were used. These were previously morphinized, then etherized, placed on an operating table, and connected from the left carotid artery to an ordinary mercury manometer for recording changes in blood pressure, which was observed as the hemorrhages were continued, and subsequently during the injection of peptone. An ordinary glass cannula was tied in the left femoral artery for making the interrupted hemorrhages. A small serrefine clip was placed on the vessel central to the cannula. When the hemorrhages were made, the clip was loosened and 1 to 2 c.c.

\*From the Department of Pharmacology, School of Medicine, Western Reserve University, Cleveland, Ohio.

of blood were allowed to escape into small vials of 4.5 cm. length and 1.2 cm. in diameter and with straight sides. Duplicate tests were made requiring the withdrawal of about 4 c.c. of blood at most into the vials plus the small quantity of blood left behind in the cannula. The vials were placed into a rack and allowed to stand at room temperature without further disturbance except for a slight and gentle tilting every 15 seconds to see whether the blood still flowed. If a movement of the meniscus was discerned the vials were replaced, but, if this was absent, the vials were completely inverted. Complete invertability without loss of contents constituted the endpoint of coagulation. In several instances it was possible to invert the vials without loss of contents almost immediately after completion of the hemorrhage, or in about 10 seconds, which was the shortest time in which all movements used in the procedure could be executed. At times the rapidity of clotting was indeed remarkable. We did not record the exact or even approximate percentages of loss of blood under the conditions of our experiment. What this was we do not know, but the absolute quantity of blood withdrawn at a given moment was small.

The results of the four experiments (see Table 5, page 205, paper by Hanzlik and Weidenthal; *Jour. Pharm. Exper. Therap.*, 1919, xiv, 189) performed by us showed the coagulation time to be invariably and markedly shortened as the hemorrhage progressed. In two of the animals, after the interrupted hemorrhages had been continued for some time, the coagulation time was somewhat prolonged, though it was still shorter than the coagulation time accompanying the first bleedings. Obviously, such results can inspire no confidence in the use of the method. Therefore, we still feel justified in suspending our work at this point, and in this we are sustained by some experiences of Professor Howell, who states as follows on page 297 of his Harvey Lecture for 1916-17 concerning his results with the intravenous and other methods of administering cephalin to dogs: "I have made a large number of experiments on dogs in which cephalin in aqueous solution was injected intravenously, intraperitoneally or subcutaneously or finally was fed by mouth. The results of these experiments have not been published since they showed *many irregularities* [italics mine] that will require further experimentation to explain."

Hamilton cites the experiments of Drinker and Drinker (*Amer. Jour. Physiol.*, 1915, xxxvi, 305, and not 1915, xxxviii, 233 as given by Hamilton) quoted by Howell, whom we quoted correctly though credited erroneously, that hemorrhage itself shortens coagulation time. Hamilton interprets the loss of blood during the hemorrhages in the experiments of Drinker and Drinker as "an approximate loss of 20 per cent of blood at one time, at another 33 per cent," but I am at a loss to find any such calculations in their paper, nor was it the object of these authors to study especially the relation of quantity of blood lost to the shortening of coagulation time. Moreover, the experiments of Drinker and Drinker were made on cats and rabbits, while our experiments were made on dogs, which precludes the possibility of making strict comparisons. It is a familiar and old observation that progressive bleeding shortens the coagulation time of blood. For instance, Gray and Lunt (*Amer. Jour. Physiol.*, 1914, xxxiv, 332) cite no less than 12 investi-



gators who have been concerned with this problem under various conditions, including very small capillary hemorrhages as from finger puncture to very large hemorrhages in animals and those accompanying surgical operations on human individuals. Whatever the cause of this phenomenon may be, it is obviously a rather variable function, and to make use of a method in which this occurs and propose it as a method of bio-assay for thromboplastic agents as Hamilton does is attempting more than we would be justified in doing.

On the contrary, Hamilton's results seem to us exceptional and to require further explanations. Two of these occur to us: (1) Selection of data and (2) manipulation of the clot. Hamilton apparently obtains his results by a process of selection which appears to us as questionable, for he states as follows on page 578 of his paper: "Animals have been rejected for testing purposes: (1) Because of having too short a coagulation time: it should not be less than 6 minutes. (2) Because of some unrecognized factors which influence the coagulation time before the agent is administered. A shortened coagulation time on the second sample of blood to one-third that of the first sample was once observed when not more than 10 c.c. had been withdrawn from a 12 kgm. dog. (3) Because of failing to react to an active sample of coagulant, whose potency had been demonstrated on 2 test animals." I doubt the ultimate value of conclusions obtained by rejecting systematically any results which do not fit into a preconceived scheme.

Undue disturbance of the blood in the vials prolongs the clotting time and may even inhibit the process of coagulation. Hamilton says on page 577 of his paper in this Journal, 1920, v. 574: "One must be careful that an apparently firm clot is not due to a skin over the surface below which the blood is fluid," an inference by which he apparently justifies the following interference with the clotting process as indicated in a previous paper by him (see page 122, *Jour. Am. Pharm. Assn.*, 1920, ix, 118) when he advises that "This should be broken so that correct observations can be made."

Until these criticisms are more satisfactorily explained we consider ourselves justified in having stopped further work with this method and we adhere to our former view, that the method employed by Hamilton, namely, testing of hemostatics on coagulation time before and after intravenous injection of these, does not justify conclusions as to comparative hemostatic efficiency of thromboplastic agents; and that the methods selected by us did justify us in concluding that hemostatic serum is inert as an accelerator of coagulation of blood and plasma *in vitro*, and as a hemostatic *in vivo*.

## A PHARMACOLOGICAL STUDY OF BENZYL BENZOATE\*

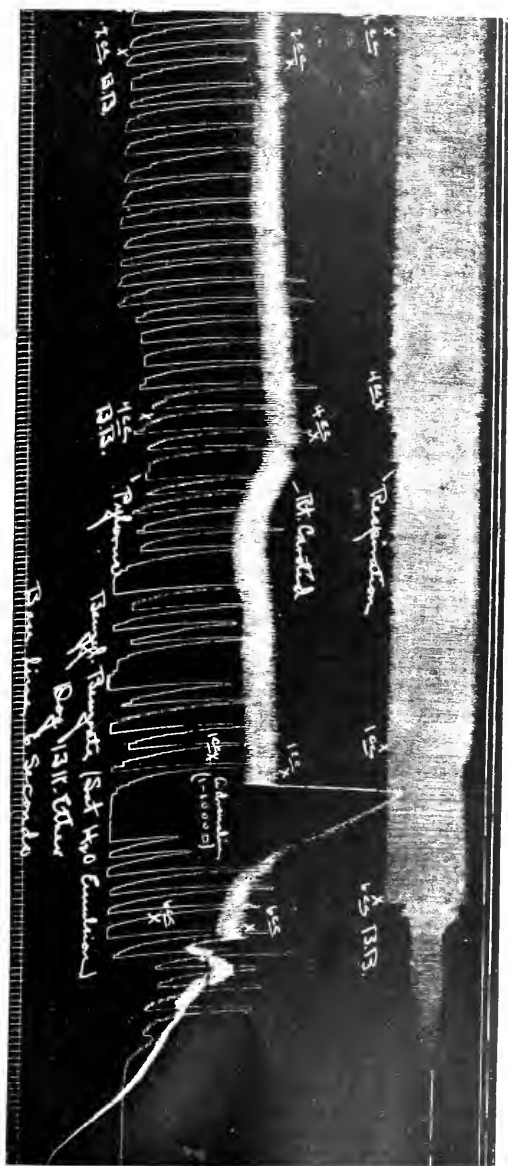
BY EDWARD C. MASON, A.B., M.D., AND CARL E. PIECK, B.S.,  
CINCINNATI, OHIO

THE recent introduction of benzyl benzoate to the Medical profession, and the apparently increasing demand for the substance have led us to undertake the present study in the hope of adding some further contribution to our knowledge of the pharmacologic action of the body. With reference to this type of benzyl derivatives it has already been stated by Macht that "inasmuch as they are practically insoluble in water, pharmacologic experiments with them could not be performed on isolated tissue *in vitro* except under special conditions." We have, of course, been confronted with this difficulty in carrying out the present experiments which have been performed entirely on the intact animal, since Macht,<sup>1</sup> in his splendid work on this subject, has probably done all that would yield valuable information through working with isolated tissues.

Three different preparations were used in the course of this work. They were sold by the following firms: Hynson, Westcott and Dunning, Baltimore; The Norwich Pharmacal Co., Norwich, New York; and Van Dyke and Co., New York. These three preparations varied somewhat in toxicity, and considerably in their property of remaining in solution. However, the pharmacologic effects produced were quite the same. By testing the action on drawn blood we found that these commercial 20 per cent preparations exercised a pronounced action on the drawn blood, and for that reason we have used the 20 per cent preparations diluted with an equal volume of water. We found this 10 per cent dilution very satisfactory for intravenous injection. In many of our records the term "saturated water emulsion" is used and is not technically correct, but refers to the 10 per cent solution just described.

In the recent medical literature<sup>2</sup> a considerable number and variety of clinical conditions are described as having been benefited, often in a very striking manner, by the use of the benzyl esters. Among these conditions may be mentioned (1) excessive peristalsis of the intestine, as in diarrhea and dysentery, (2) intestinal colic and enterospasm, (3) pylorospasm, (4) spastic constipation in which there was a tonic spastic condition of the intestine, (5) biliary colic, (6) ureteral or renal colic, (7) vesical spasm of the urinary bladder, (8) spasmodic pains originating from the contraction of the seminal vesicles, (9) uterine colic, (10) arterial spasm, including hypertension or high blood pressure, (11) bronchial spasm. It has been our aim in this study to shed some light, if possible, on the mechanism by which these conditions are relieved, and to ascertain the concentration of the drug in the blood necessary to produce the desired results.

\*From the Pharmacological Laboratory of the University of Cincinnati Medical School, Cincinnati, Ohio.



It will be noted that the first four clinical conditions listed above refer to increased activity or increased tonus of the intestinal tract. We have therefore felt it desirable to study the nature of the relaxation produced by the benzyl benzoate in these conditions. Fig. 1 shows the respiration, right carotid pressure and pylorus contractions in a dog weighing 13 kilos. Near the beginning (left) of the tracing 2 c.c. of benzyl benzoate solution was injected into the femoral vein. This produced no appreciable change in the rate or amplitude of either the pyloric contractions or the respiration. There is perhaps

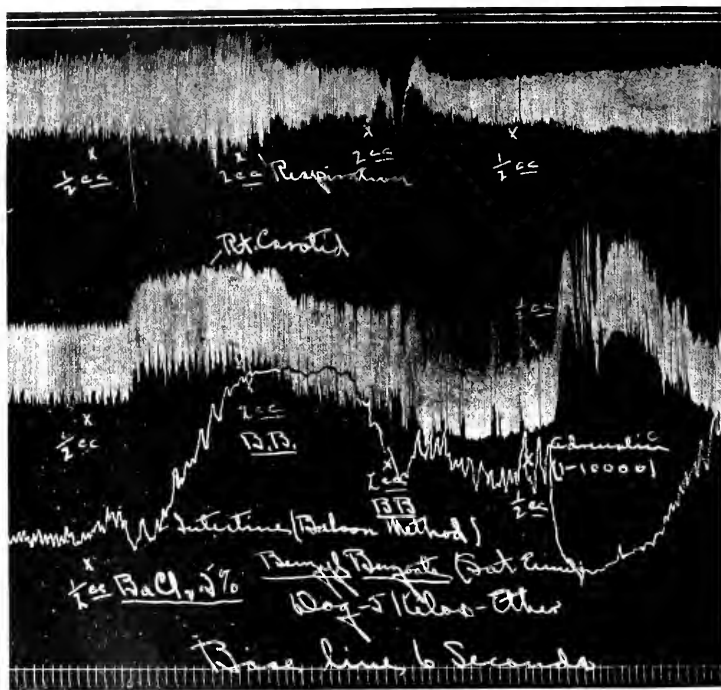


Fig. 2.

a slight change in the blood pressure tracing. Following this 4 c.c. of the benzyl benzoate solution was injected and this produced a depression of the respiration, an obvious fall in blood pressure and perhaps a very slight inhibitory effect on the pyloric contractions. An injection of 1 c.c. of adrenaline (1:10,000) was now given as a check on the technic of the experiment. This produced a very obvious, but rather brief, inhibition of the pyloric contractions. When the animal again returned to normal a further injection of 6 c.c. of benzyl benzoate was given. After a brief interval this dosage produced

complete relaxation of the pylorus, but simultaneously it caused the death of the animal, apparently by central respiratory paralysis. This tracing shows very well the progressive effects of small, medium and large doses of the drug. The marked slowing of the heart following the 4 c.c. injection should be noted.

In order to get further information regarding the action of the drug on the intestinal walls we carried out a series of experiments as illustrated in Fig. 2. In this case a small rubber balloon (finger cot) filled with water was placed within the intestinal lumen and connected by rubber tubing with a burette which carried a stopper in the upper end. From the stopper a rubber

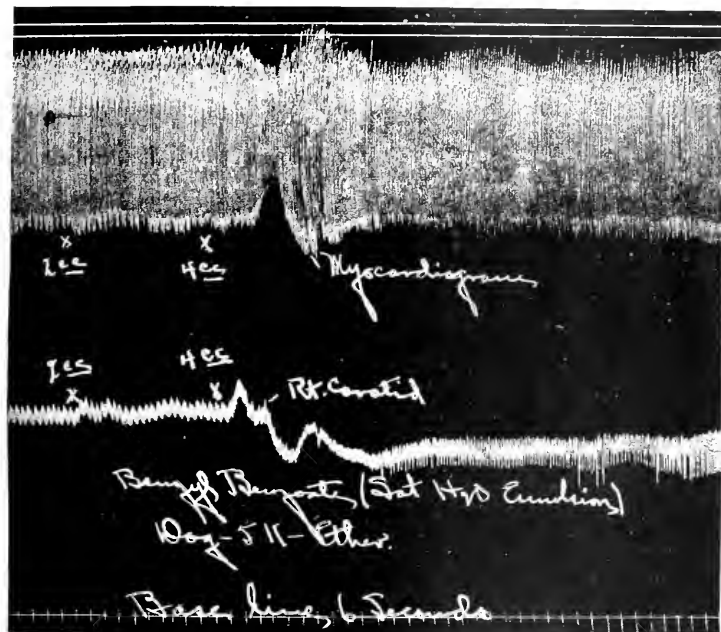


Fig. 3.

tube led to the recording tambour. The water filled the balloon and reached about two inches up in the burette, the upper part of which was filled with air. Thus contractions which lessened the lumen of the intestine caused the writing point of the tambour to rise, and relaxation of the gut showed a fall in the kymograph tracing. In the beginning a small dose (1 $\frac{1}{2}$  c.c. of .5 per cent solution) of barium chloride was injected to stir up marked contractions of the intestine. The marked rise in tone of the intestine is well shown in the tracing. When the circular musculature of the gut was well contracted an injection of 2 c.c. of benzyl benzoate was given. This depressed the respira-

tion and slightly lowered the blood-pressure, but produced no immediate change in the tone of the intestine. After an interval of about one minute, however, there occurred a relaxation of the intestine and at this point a further injection of 2 c.c. of the drug was given. This apparently slightly increased the tonus of the intestine and was followed by a series of small, irregu-

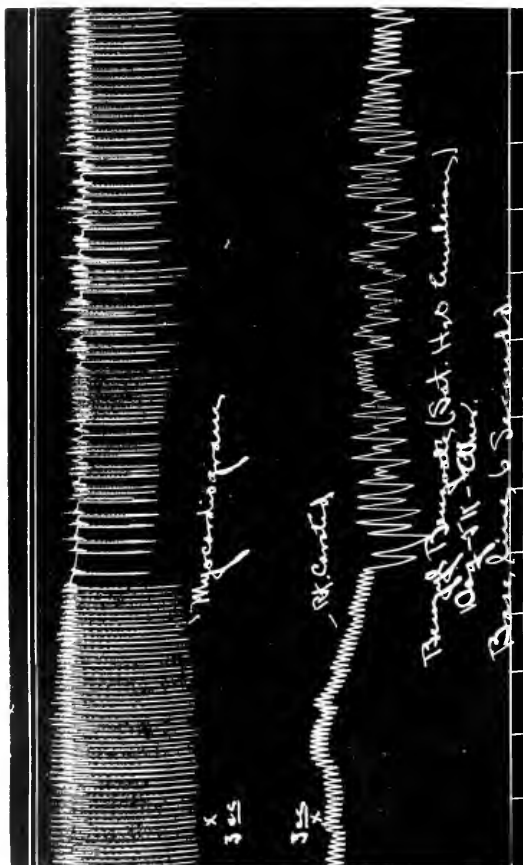


Fig. 4.

lar contractions. As a check on the technic of the experiment an injection of  $\frac{1}{2}$  c.c. of adrenalin was finally given. This produced an immediate and complete relaxation of the gut. It will be noted that the second injection of benzyl benzoate greatly depressed the respiration, but both injections of benzyl benzoate taken together were very much less effective in lowering the tone

of the intestinal musculature than was the response of 1-10,000 units of atropine solution.

The injection of a sufficiently large dose of 0.5 ml benzocaine (20% solution) produced a prompt, pronounced and prolonged fall of blood pressure. From 0.5 to 1.0 ml of

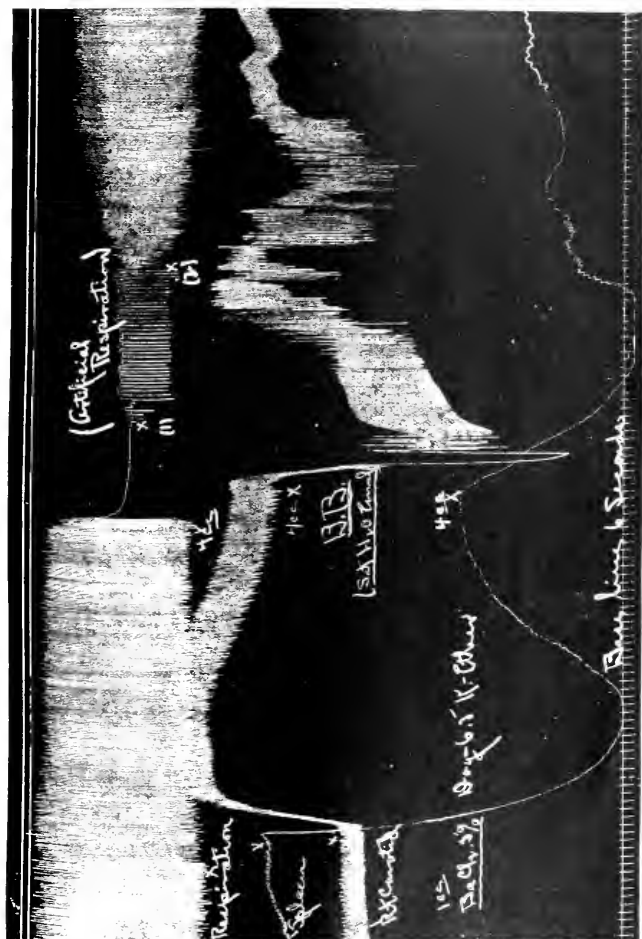


Fig. 5.

of the blood pressure curve produced, we early conceived the idea that the heart played an important part in this lowering of the arterial tension. Fig. 3 shows a tracing taken directly from the heart (upper curve; myocardiograph)

and the right carotid pressure tracing. It will be noted that 2 c.c. of benzyl benzoate gave little or no change, but that 4 c.c. produced the characteristic fall in blood pressure. It is obvious that this fall in pressure is closely associated with the changes in the amplitude and force of the heart beat. But in order to analyze this point still further we present Fig. 4 in which the drum



Fig. 6.

was made to revolve much more *rapidly* than in Fig. 3. Fig. 4 shows that 3 c.c. of the drug was followed by a fall in blood pressure, but that the heart at the same time became weak and after an interval came near stopping in diastole. After a prolonged series of feeble, irregular beats, however, the heart again recovered slightly, and the blood pressure finally became somewhat more



regular. These changes in the blood pressure are obviously due to a relaxing action of the drug on the heart. It should be noted that this animal was receiving artificial respiration.

Fig. 5 shows the action of benzyl benzoate on the respiration, spleen volume and blood pressure of a dog weighing 6.5 kilos. In order to produce a tonus in the spleen an injection of 1 c.c. of .5 per cent barium chloride was given at the beginning of the tracing. The contraction of the spleen became very marked and this was followed after a time by some relaxation. Four c.c. of benzyl benzoate was now injected in order to see whether or not the drug would relax the spleen. It will be noted that the spleen-concometer tracing again dips

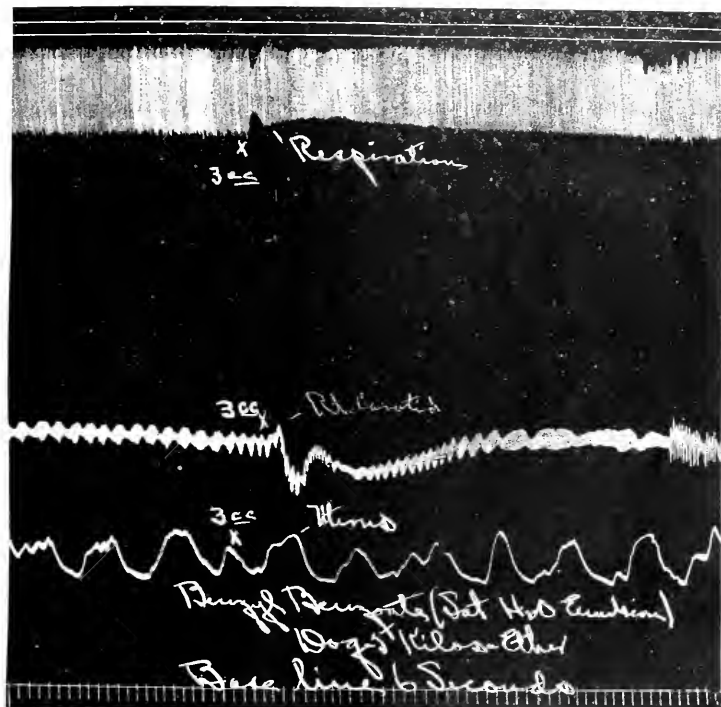


Fig. 7

even lower than it did following the marked contraction produced by the barium chloride. But the causes of these two spleen contractions are, however, exactly the opposite of each other. In the first, the shrinkage of the spleen volume represents an *active contraction* of smooth muscle within the spleen. The second contraction is *passive*, and is due to the profound fall in blood

pressure, and perhaps, somewhat to the asphyxia which followed the injection of the benzyl benzoate. Artificial respiration was started at "1" and stopped at "2." Although the blood pressure again returned to a high tension (continued action of the barium) and the spleen tracing also marked a low level (also barium contraction), still there is no evidence that the benzyl benzoate exercised any relaxing action on the spleen, although the dosage was perhaps sufficient to have killed the animal if artificial respiration had not been given. Under artificial respiration animals can withstand very much larger doses of the drug without dying, than is possible under natural respiration. This indicates that the animals do not die of thrombosis as might be suspected from the rather unsatisfactory character of the drug for intravenous injection. The

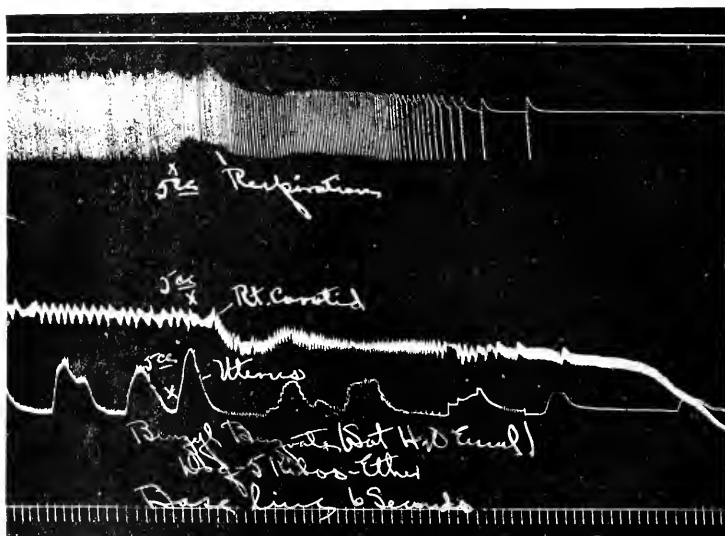


Fig. 8.

final recovery of the heart and circulation, if artificial respiration be maintained, shows that no permanent thrombi are lodged in any vitally important vascular areas.

Fig. 6 shows the respiration, kidney volume (oncometer) and blood pressure. In the beginning 4 c.c. of benzyl benzoate solution was injected. Slight effects were produced in all three tracings. The small shrinkage in kidney volume is obviously secondary, and due to the fall in blood pressure. If the arterioles themselves in the kidney had dilated the volume record would have risen, as occurs after the constriction produced by the 1 c.c. injection of adrenalin (at the center of the tracing) begins to wear off. The shrinkage of the kidney volume after the adrenalin is active and is due to the adrenalin stimulating

the myoneural junctions of the vasoconstrictor nerves in the renal arterioles. Following the adrenaline a further dose of 7 c.c. of benzyl benzoate was given. The results of this were exactly analogous to those produced by the 4 c.c. but were correspondingly more pronounced.

The recently suggested use of benzyl benzoate in clinical conditions presumably dependent on excessive or abnormal contraction of the uterus indicated that the drug would probably produce relaxation of this organ. Figs.



Fig.

7 and 8 show the results we have obtained in the study of the action of the drug on this organ. In Fig. 7 an injection of 3 c.c. was made and produced very obvious results on the respiration and blood pressure. On the uterus, however, the results are very slight. Fig. 8 also shows the effects of an injection of 5 c.c. of the drug. This dosage finally stopped the uterine contractions, but not until the animal had died. Obviously one could not use such large amounts clinically. In these tracings the uterus remained *in situ* and great care was used not to disturb its innervation or blood supply, and to keep the organ warm and moist by closing the abdominal wall and covering the recording apparatus with the intestines (see Jour. Lab. and Clin. Med., 1917, iii, 63). In regard to these two records, however, it should be stated that it is often

difficult to secure entirely satisfactory tracings of the uterus *in situ* in dogs, and we should not be inclined to lay too great emphasis on these observations without checking the results by a considerable number of experiments on animals of different species, which we have not as yet had an opportunity to do. In our present experiments the uterus had previously been roused to increased activity by the injection of pituitrin.

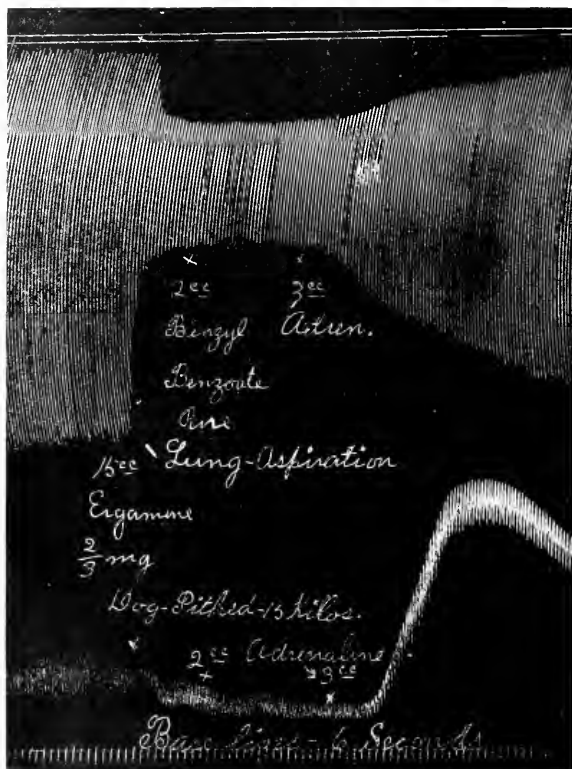


Fig. 10.

One of the most important clinical uses suggested for benzyl benzoate is in the treatment of bronchial asthma.<sup>4</sup> Fig. 9 represents two tracings taken from separate dogs and mounted together. They show the action in spinal dogs of histamine (3-iminazolyethylamine, "ergamine"), benzyl benzoate, codeine and adrenaline on the bronchioles as recorded by a special aspiration method (see Jour. Pharmacol. and Exper. Therap., 1914, vi, 57; also Jackson's



Experimental Pharmacology, C. V. Mosby Co., 1917, St. Louis, p. 287). In these tracings (also Figs. 10 and 11) a shortening of the amplitude of the lung record means contraction of the bronchioles, and increase in the amplitude of the lung tracing shows dilatation of the bronchioles. In Fig. 9 the left hand record shows a contraction of the bronchioles produced by the intravenous injection of  $2\frac{1}{2}$  milligram of ergamine. This led to a bronchial contrac-

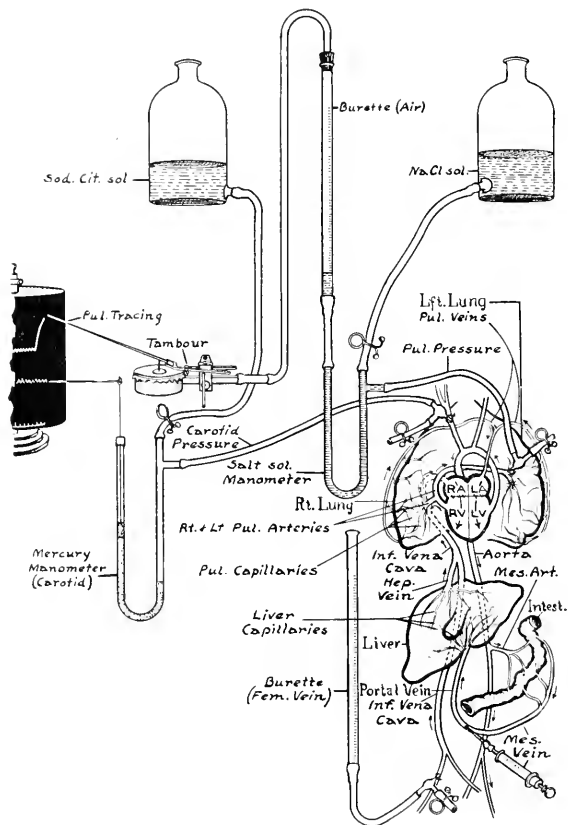


Fig. 12.

tion which 10 c.c. and, later 50 c.c. of an aqueous solution of benzyl benzoate did not relax. An injection of adrenaline (2 c.c.) produced prompt bronchial dilatation. In the right hand tracing eodine was used to produce the initial bronchial contraction and then 3 c.c. of the 20 per cent benzoate made up in 75 per cent alcohol was injected intravenously. The dog weighed 8 kilos and

it would appear that this dosage should certainly have caused dilatation of the bronchioles. This did not occur, however, and as a further check on the technique of the experiment 2 c.c. of adrenalin was injected. A bronchial dilatation followed although the heart stopped beating (from the effects of the codeine and benzyl benzoate). Fig. 10 shows that 2 c.c. of *pure* benzyl benzoate (made by the Harmer Laboratories) did not produce the slightest indication of a bronchial dilatation following a contraction produced by "ergamine." Adrenaline, however, caused a marked dilatation. This seems to indicate definitely that benzyl benzoate does not cause a bronchodilatation in intact, pithed dogs under the conditions obtaining in such experiments as we have here carried out. Fig. 11 shows three separate tracings mounted together. From the legends it will be seen that benzyl benzoate did not produce satisfactory bronchodilatation in either instance.

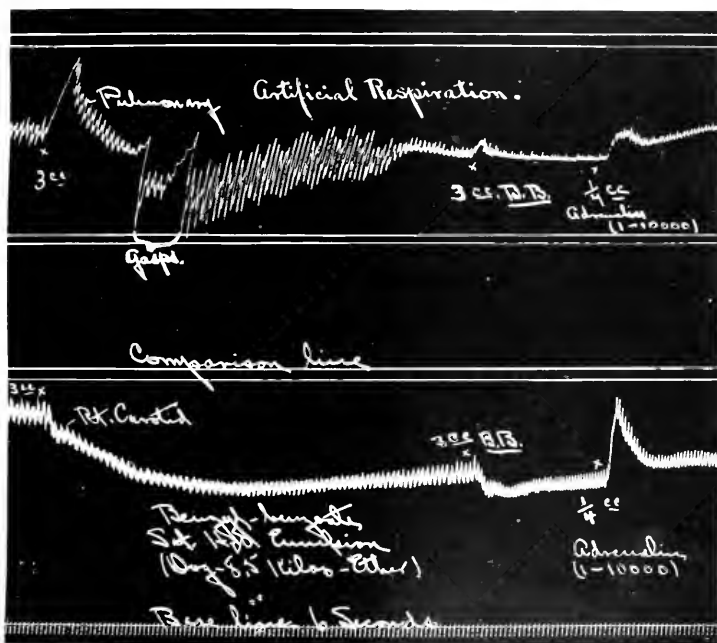


Fig. 13.

The possibility that benzyl benzoate might be used clinically in pulmonary hemorrhage in cases of tuberculosis led us to investigate the action of the drug on the pulmonary blood pressure. The method we have used for recording the pulmonary arterial pressure is indicated in Fig. 12. (See Jour. Lab. and Clin. Med., 1920, vi, 11.) In the diagram it will be seen that a cannula tied into

the left pulmonary artery connects with a water manometer, the distal end of which is joined to the lower end of a burette. The manometer and tubes to the artery (and the cannula) are filled with normal salt solution. The upper end of the burette is connected with a recording tambour which writes on the

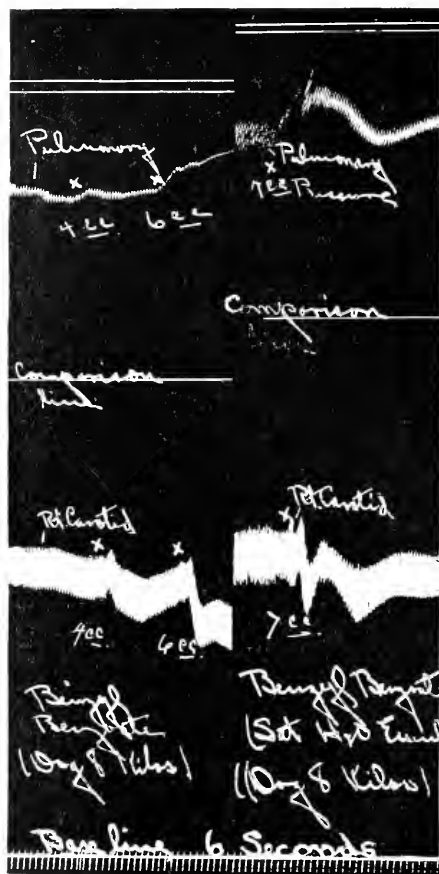


Fig. 14.

drum. The tambour, the upper part of the burette and the connecting tubes are filled with air. By this method the pulmonary blood pressure record represents a magnification about 150 times greater than would be recorded by a mercury manometer. The variations in the pulmonary tracings should, there-



fore, be reduced about 150 times in amplitude in order to compare them directly with the associated carotid tracings which, in our records, were made with a mercury manometer. Fig. 13 shows the results we have observed on the pulmonary arterial pressure. Near the beginning of the tracing 3 c.c. of benzyl benzoate was injected and a typical, prolonged fall in the carotid pressure, was obtained. In the pulmonary pressure, however, there was produced at first a sharp temporary rise which was succeeded by a few gasping movements. But on the average there was extremely little variation in the pulmonary pressure, either in the way of a rise or a fall. Later a 3 c.c. injection of benzyl benzoate was given again. The fall produced in the carotid pressure was again quite typical, but the pulmonary pressure showed only a very slight, transient rise. A small injection of adrenaline was finally given in order to check up the accuracy of the technic in the experiment. Fig. 14 shows two short pulmonary tracings (mounted together). Here again the drug produced only the slightest changes in the pulmonary pressure. In studying these tracings one must, of course, constantly bear in mind the greatly increased magnification of the pulmonary over the carotid records. It is obvious that if the drug should act in clinical cases in a manner at all analogous to that in which it has behaved under these experimental conditions, it could be of no use in the matter of treatment of hemoptysis in pulmonary tuberculosis.

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- <sup>3</sup>Litzenberg, Jennings C.: Jour. Am. Med. Assn., Aug. 23, 1919, pp. 601-603.
- <sup>4</sup>Macht, David I.: Southern Med. Jour., July, 1919, xii, No. 7, p. 367.

# A REPORT OF AN EPIDEMIC OF INFLUENZA IN AN ARMY POST OF THE AMERICAN EXPEDITIONARY FORCES IN FRANCE

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## A. INTRODUCTION

THE outbreak of influenza which forms the subject of this paper was described in an official report by one of us (Chesney) to the Chief Surgeon, American Expeditionary Forces, France. At the instance of the Surgeon General that report has been revised for publication and forms the basis of this paper. It was thought that a report of this particular epidemic would be of interest in view of the fact that it seemed to throw some light on one or two problems related to the epidemiology of influenza, more especially the question as to whether or not the virus of influenza acquires increased virulence by successive passage through human hosts.

## B. SITE OF THE EPIDEMIC

The outbreak which it is proposed to describe occurred in the Post of A. P. O. 704, American Expeditionary Forces, France. This post was a permanent artillery camp of the French Army, located at LeValdahon in the department of the Doubs. It comprises a tract of rolling land large enough to provide sufficient room for artillery range, and in addition possesses a number of permanent two-story barracks buildings of cement construction. The use of this camp was loaned to the American Army and after it had been taken over by the latter a number of wooden barrack buildings were erected for purposes of instruction and for additional barrack and stabling space.

During the summer and autumn of 1918 American artillery brigades were ordered to this post for periods of from four to six weeks for instruction. It frequently happened that while one brigade occupied the post and was receiving instruction another brigade was billeted in neighboring towns and was awaiting the opportunity to enter the post for the same purpose. It was the custom of one brigade to enter the post as soon as its predecessor had left. Thus every four to six weeks there occurred a marked change in the population of the post.

In addition to the brigades which occupied the post there was a permanent post personnel constantly present which consisted of troops belonging to the Cavalry arm, the Engineer Corps, Quartermaster Corps, and Medical Corps, which were engaged in the administration of the post. The permanent personnel amounted to about 500 and was fairly constant. The total population of the post when a brigade was billeted in it varied from 3500 to 5000. The post hospital, Camp Hospital No. 12 received patients from all the organ-

izations in the post as well as from those in the surrounding billeting area. One of us (Snow) was Commanding Officer of the hospital during the period of the epidemic and Post Surgeon from about August 1st, the other (Chesney) was detailed to make a special study of the epidemic.

The frequent changes in the population of the post brought about by the short stay of each brigade exercised considerable influence upon the course of the epidemic. Indeed the history of the epidemic resolves itself into distinct periods corresponding to the various brigades which entered the post and it will be profitable to approach the events from that point of view.

### C. HISTORY OF THE EPIDEMIC

#### 1. First Period, June to July 27th, 1918

The first period covers the duration of the stay of the 5th Field Artillery Brigade in the post. During this interval a respiratory infection diagnosed as influenza made its appearance in troops billeted there. It was largely confined to troops belonging to the 5th Brigade. Thus of 77 cases diagnosed as influenza, bronchopneumonia or lobar pneumonia admitted to the post hospital between July 1st and July 27th from organizations actually barracked in the post, 68 developed in organizations of the 5th Brigade. In Table I is shown the distribution of these cases among the commands in the post.

TABLE I

DISTRIBUTION OF CASES OF RESPIRATORY DISEASE IN ORGANIZATIONS BARRACKED IN POST OF  
A. P. O. 704, JULY 1-27, 1918

ORGANIZATION	NUMBER OF CASES ADMITTED TO HOSPITAL
<i>5th Artillery Brigade</i>	
Brig. Hdqrs. Co.	17
5th Trench Mortar Battery	12
<i>19th Field Artillery Reg't</i>	
Battery A.	2
Battery B.	2
Battery C.	2
Battery D.	1
Officers	1
<i>20th Field Artillery</i>	
Regt.	
Headquarters Co.	3
Supply Co.	1
Sanitary Detachment	1
Battery A.	1
Battery B.	4
Battery C.	5
Battery D.	2
Battery E.	2
Battery F.	2
Officers	3
<i>21st Field Artillery Regiment</i>	
Headquarters Co.	1
Battery B.	1
Battery C.	2
Battery E.	2
Officers	1
<i>Permanent Post Organizations</i>	
Troop C. 2nd Cavalry	5
Medical Detachment Camp Hospital No. 12	2
Unattached Officers	2

In compiling these statistics no differentiation has been made between cases of influenza, acute bronchitis, bronchopneumonia or lobar pneumonia. They are all grouped under the heading "Acute Respiratory Disease." As a matter of fact most of the cases were cases of influenza.

From Table I it is seen that certain organizations were affected more than others, notably Headquarters Company of the brigade and the 5th Trench Mortar Battery. The disease was relatively mild at that time. Just how it made its way into the post is uncertain, but it was known to be prevalent among the civilian population of Besaneon, a city located some 30 kilometers away, and the troops were permitted to visit that city on pass. During the month of July, while the 5th Brigade occupied the post the 58th Field Artillery Brigade occupied billets in surrounding towns and was waiting to enter the post for its period of training. The withdrawal of the 5th Brigade marks the end of the first period of the epidemic and the beginning of the second.

## 2. SECOND PERIOD, JULY 27TH TO AUG. 23RD

The second period, lasting from July 27th to August 23rd, represents the duration of stay of the 58th Field Artillery Brigade in the post. This Brigade was composed of the 122nd, 123rd, and 124th Field Artillery Regiments, the 108th Ammunition Train, the 108th Trench Mortar Battery and the 108th Motor Ordnance Repair Shop. During the time that these organizations occupied billets in the vicinity of the post, a period of four weeks before their entry, no cases of influenza, bronchitis or pneumonia occurred in them.

The 123rd and 124th F. A. Regiments began to enter the post on July 27th, and about three days were consumed in the process of moving in. The 122nd Regiment did not enter the post at all, but remained in billets in a town about one kilometer distant and sent men to the ranges of the post every day for instruction in firing. The troops of the 123rd and 124th Regiments occupied barracks at the southwestern end of the post and slept upon mattresses and pillows that had been used by outgoing troops of the preceding brigade. No general attempt was made to clean these barracks before the entry of the new brigade.

Several days after the 58th Brigade had entered the post, cases of influenza began to appear in units of that brigade and the number of such cases increased rapidly. The distribution, among the various commands in the post, of cases of acute respiratory disease that occurred between July 27th and August 23rd inclusive, and were admitted to the post hospital, is shown in Table II.

Table II shows that of all cases of acute respiratory disease admitted to the post hospital during the period of July 27th to Aug. 23rd, the great majority came from the 123rd and 124th Field Artillery regiments. In Figs. 1, 2, and 3 are exhibited curves which show the daily number of cases of acute respiratory disease admitted to hospital from these organizations, together with those from the 122nd regiment, during the entire time of their stay in the post as well as for the previous four weeks during which they were in towns in the neighborhood. These curves show that there were no cases of influenza

or other respiratory infection in these regiments during the first four weeks of July, while all three were billeted in nearby towns and that the 123rd and 124th F. A. regiments, which occupied the camp during the last few days of July and the first three weeks of August, had a number of cases of influenza following their entrance into the post, whereas the 122nd F. A., which remained

TABLE II

DISTRIBUTION OF CASES OF ACUTE RESPIRATORY DISEASE OCCURRING IN COMMANDS BAR-  
RACKED IN POST A. P. O. 704, A. E. F. DURING JULY 27th TO AUG. 23rd, 1918

ORGANIZATION	NUMBER OF CASES ADMITTED TO HOSPITAL
<i>58th Field Artillery</i>	
Brigade Headquarters Company	0
108th Ammunition Train	1
108th Trench Mortar Battery	5
108th Motor Ord. Repair Shop	2
<i>123rd Field Artillery Reg't</i>	
Headquarters Co.	5
Supply Co.	6
Ordnance Det.	2
Sanitary Det.	5
Battery A.	11
Battery B.	3
Battery C.	3
Battery D.	19
Battery E.	6
Battery F.	6
Officers	7
<i>124th Field Artillery</i>	
Headquarters Co.	21
Supply Co.	0
Ordnance Det.	0
Sanitary Det.	10
Battery A.	14
Battery B.	2
Battery C.	12
Battery D.	2
Battery E.	33
Battery F.	30
Officers	4
<i>Sixth Field Artillery Brigade</i>	
*Brigade Headquarters	2
*6th Trench Mortar Battery	1
*Headquarters F. A.	1
*Headquarters Company 11th F. A.	1
*Supply Company 11th F. A.	1
*Ordnance Detachment 11th F. A.	1
*Battery E. 11th F. A.	4
*Battery F. 11th F. A.	2
<i>Post Organizations</i>	
2nd Cavalry	2
309 Q. M. C.	10
Post Engineers	4
Post Ordnance	3
Post Signallers	2
Medical Detachment Camp Hospital No. 12	10
3rd Veterinary Hospital	1
800th Aero Squadron	4
14th Balloon Squadron	1
Officers Unattached	2
	<hr/> 252 <hr/>

\*These organizations were sent into the post in advance of the Sixth Brigade itself for preliminary instruction.

billeted in LeValdahan, a kilometer away, had only six cases of influenza during the entire time, three of which could be fairly definitely traced to the post. This last regiment, which remained outside the camp but sent men to the ranges daily for instruction, served therefore as a satisfactory control

Fig. 1

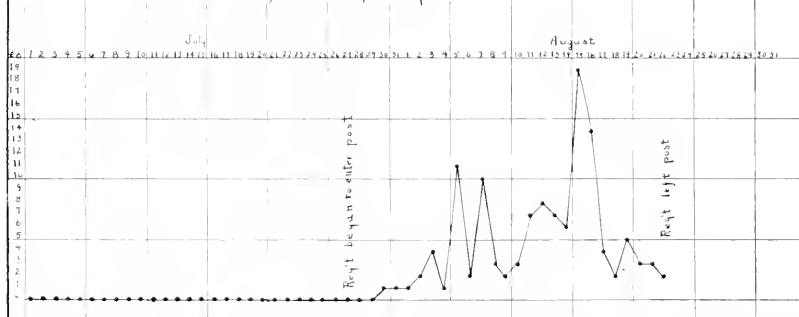
Incidence of Acute Respiratory Disease in 124<sup>th</sup> F.A.

Fig. 2

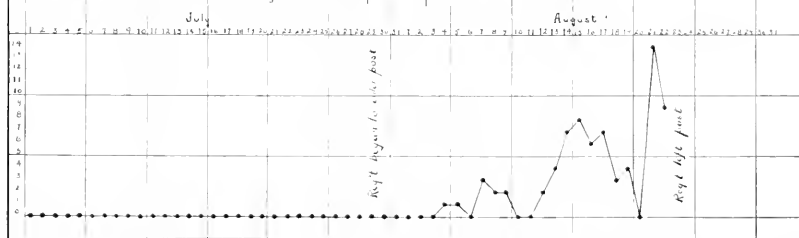
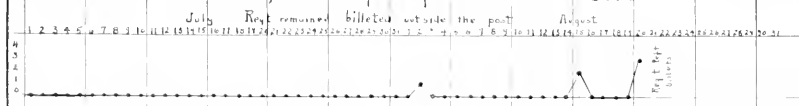
Incidence of Acute Respiratory Disease in 123<sup>rd</sup> F.A.

Fig. 3

Incidence of Acute Respiratory Disease in 122<sup>nd</sup> F.A.

for the regiments which entered the camp. There can be little doubt that the organizations of the 58th Brigade which underwent an epidemic of influenza while in the Post of A. P. O. 704, A. E. F., became infected as a direct result of moving into an infected place. They did not bring the infection with them.

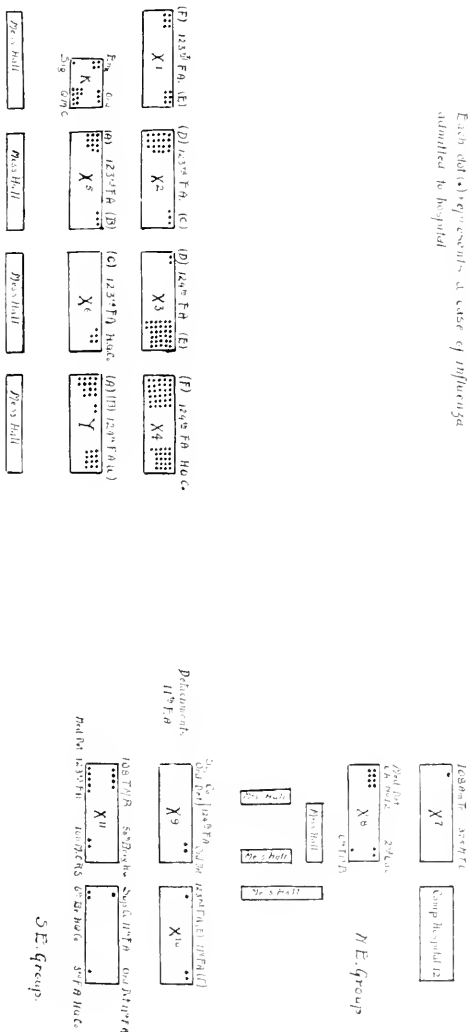
The total number of cases of respiratory infection admitted to the hospital from those organizations of the 58th Brigade located in the post

## Fig 4

DIAGRAM of BARRACKS AT APO # 733 ILLUSTRATING LOCATION of INFLUENZA CASES IN CAMP

*During PERIOD of July 27<sup>th</sup> - Aug 23<sup>rd</sup> 1911.*

*Each doctor requires a case of influenza admitted to hospital*



amounted to 200. The total strength of these same organizations on August 1st was approximately 3000, so that during their stay in the post about 6.5 per cent of the troops belonging to them were rendered unfit for duty on account of acute respiratory disease.

A spot map (see Fig. 4) designed to show the location of cases in camp, demonstrated that the disease was confined largely to the southwestern group of barracks and that there were relatively few cases in the northeastern and southeastern groups. The southwestern group of barracks which was occupied by the 123rd and 124th Regiments, as well as by some of the permanent personnel of the post, had previously been occupied by those organizations of the preceding (Fifth) brigade in which influenza had been most prevalent. The northeastern group was occupied by some permanent post organizations among which a few cases only occurred and the southeastern group was occupied by some organizations of the 58th Brigade as well as by some detachments of the 6th Brigade which had been sent into the post in advance of the brigade itself for preliminary instruction.

Once the epidemic had commenced in the 58th Brigade it assumed the characteristics of a battery infection, being fairly well restricted to certain batteries, while other batteries were almost entirely free from the infection. (See Table II.) It appears probable that the disease was spread among the men by direct contact in the barracks, where there was ample opportunity for such contact to occur. Inspection of the barracks showed that the beds were frequently less than one foot apart and that no consistent attempt at head and foot arrangement was made. The estimated floor space per man varied from 31 to 50 square feet, and was usually less than 40 square feet.

On August 22nd the 124th F. A. vacated its barracks and on the following day the 123rd left the post. Both of these regiments swept out their barracks before leaving. Their departure marks the end of the second period of the epidemic.

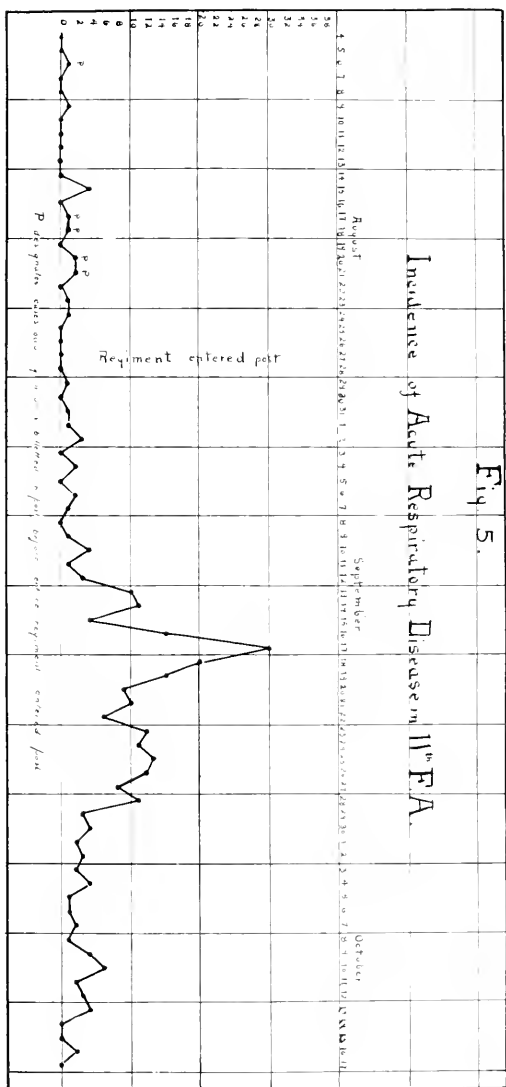
### 3. THIRD PERIOD, AUGUST 23RD TO NOVEMBER 8TH, 1918

The third period may be said to extend from August 23rd, the date of departure of the 58th Brigade, to November 8th, the date of departure of the last of the brigades sent to the post for instruction during the Summer and Autumn of 1918. This period represents the duration of stay of the 6th and 156th Artillery Brigade in the post.

During the first three weeks of August, while the 58th Brigade was occupying barracks in the post, the 6th Field Artillery Brigade was billeted in nearby towns, awaiting its turn to enter the post for instruction. Several units from the brigade were actually sent into the post in advance of the brigade itself and occupied barracks in the southeastern portion of the post during the time that the 58th Brigade was having its epidemic. Table II shows that thirteen cases of acute respiratory infection occurred among these advance units of the 6th Brigade after they entered the post. The remaining units of the brigade during their stay in billets outside the camp were almost entirely free from acute respiratory disease, not more than twelve cases being



admitted to the hospital from these organizations during the period preceding their entry into the post.



When it became known that the 58th Brigade was about to leave the post and that the 6th was to enter, efforts were made by the Post Surgeon at that time (Snow), assisted by the medical officers of the 6th Brigade, to prevent the occurrence of an epidemic of influenza in the latter brigade. The situation was explained to the Commanding General of the 6th Brigade and it was recommended to him that his brigade be kept in billets in the surrounding towns rather than be permitted to enter the post, or, if the brigade did enter the post, that the men be put under tents rather than in the barracks of the post. In view of the experience with the 58th Brigade it was predicted that if the 6th Brigade entered the barracks vacated by the former brigade the latter would, in all probability, experience an epidemic of influenza as severe as, or even more severe than, that undergone by the 58th Brigade. These recommendations were not accepted. However, it was decided that a week should be permitted to elapse between the departure of the 58th and the entry of the 6th Brigade into the post, and that during that interval the barracks should be thoroughly scrubbed with soap and water and whitewashed, and the pillows and mattresses aired in the sunshine. Orders were issued to the incoming troops to use sacks filled with hay or straw for bedding and not use bedding that had been used by the outgoing brigade. The men were supposed also to be billeted in such a manner that each had at least 40 square feet of floor space.

Immediately following the departure of the 58th Brigade detachments from the 6th Brigade began to carry out the prescribed cleansing measures in the southwestern group of barracks. All beds, bedding and movable fixtures were taken out doors and left in the sunshine for several days.

The walls were washed down with soapy water applied with brooms, the floors were then scrubbed with soap and water and some of the rooms were whitewashed. The work in the southwestern group of barracks was performed by detachments from the 3rd and 78th Field Artillery regiments, which had been selected to occupy those particular barracks. The southeastern barracks were not cleaned by the regiment (11th F. A.) which was to occupy them until several days after that regiment had moved into them. Two batteries of the regiment together with some other detachments from the 6th Brigade had moved into these barracks several weeks previously and thirteen cases of influenza had developed in these units after their entry (See Table II).

On August 27th the 6th Brigade entered the post. During the fortnight following the entrance of the brigade into the post some cases of influenza made their appearance in the three regiments constituting the brigade, but the cases were scattered and the incidence was not such as to constitute a real epidemic until the 10th of September when a large number of men from the 3rd F. A. regiment suddenly came down with the disease. From that time on cases developed with great rapidity in the regiment and two or three days later the disease was epidemic in the other two regiments of the brigade. The permanent post organizations continued to contribute cases of influenza during this time. In Table III is shown the distribution, among the various

commands in the post, of cases of acute respiratory disease admitted to the post hospital during the stay of the 6th Brigade in the post.

Table III shows that of the cases of acute respiratory disease admitted to the post hospital from organizations barracked in the post during the period August 24th to October 21st, 1463 developed in organizations belong-

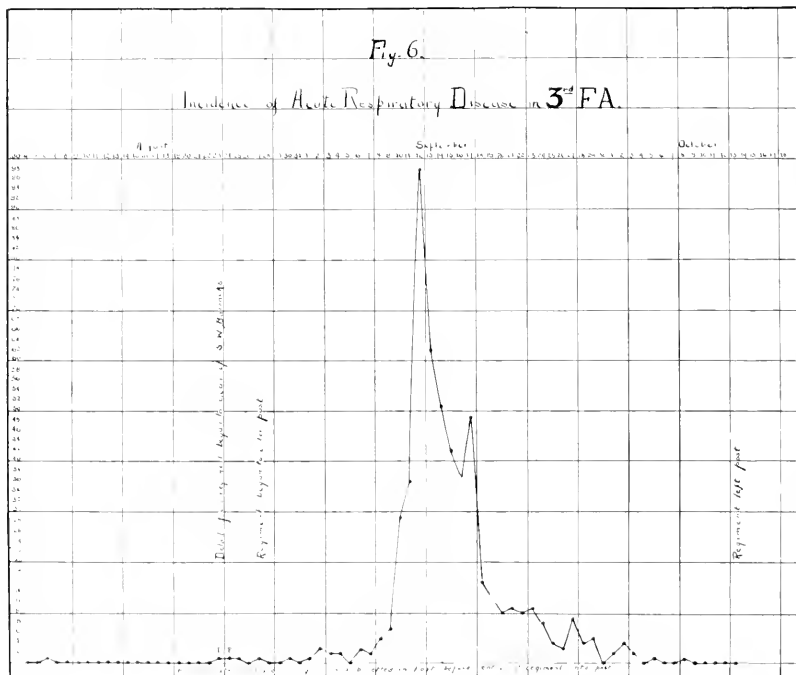
TABLE III

DISTRIBUTION OF CASES OF ACUTE RESPIRATORY DISEASE OCCURRING IN COMMANDS BARRACKED IN THE POST OF A. P. O. 704, A. E. F. DURING THE PERIOD OF AUGUST 24th TO OCTOBER 21st, 1918

ORGANIZATION	NUMBER OF CASES ADMITTED TO HOSPITAL
<i>6th Artillery Brigade</i>	
Brigade Headquarters Co.	9
Brigade Casual Detachment	138
<i>3rd Field Artillery</i>	
Headquarters Co.	71
Supply Co.	60
Ordnance Det.	6
Sanitary Det.	13
Battery A.	74
Battery B.	52
Battery C.	140
Battery D.	63
Battery E.	30
Battery F.	15
Officers	16
<i>11th Field Artillery</i>	
Headquarters Co.	46
Supply Co.	7
Ordnance Det.	4
Sanitary Det.	12
Battery A.	31
Battery B.	28
Battery C.	30
Battery D.	15
Battery E.	55
Battery F.	27
Officers	10
<i>78th Field Artillery Regiment</i>	
Headquarters	49
Supply Co.	50
Ordnance Det.	4
Sanitary Det.	7
Battery A.	103
Battery B.	78
Battery C.	22
Battery D.	34
Battery E.	25
Battery F.	120
Officers	19
<i>Post Organization</i>	
2nd Cavalry	4
309 Q. M. C.	14
Post Ordnance	4
Post Headquarters	10
Camp Hospital	51
Officers Unattached	8
3rd Vet. Hospital	45
375th M. T. C.	1
800th Aero Squad	25
14th Balloon Sq.	3
304th Remount	3
323rd Labor Batt'n	13

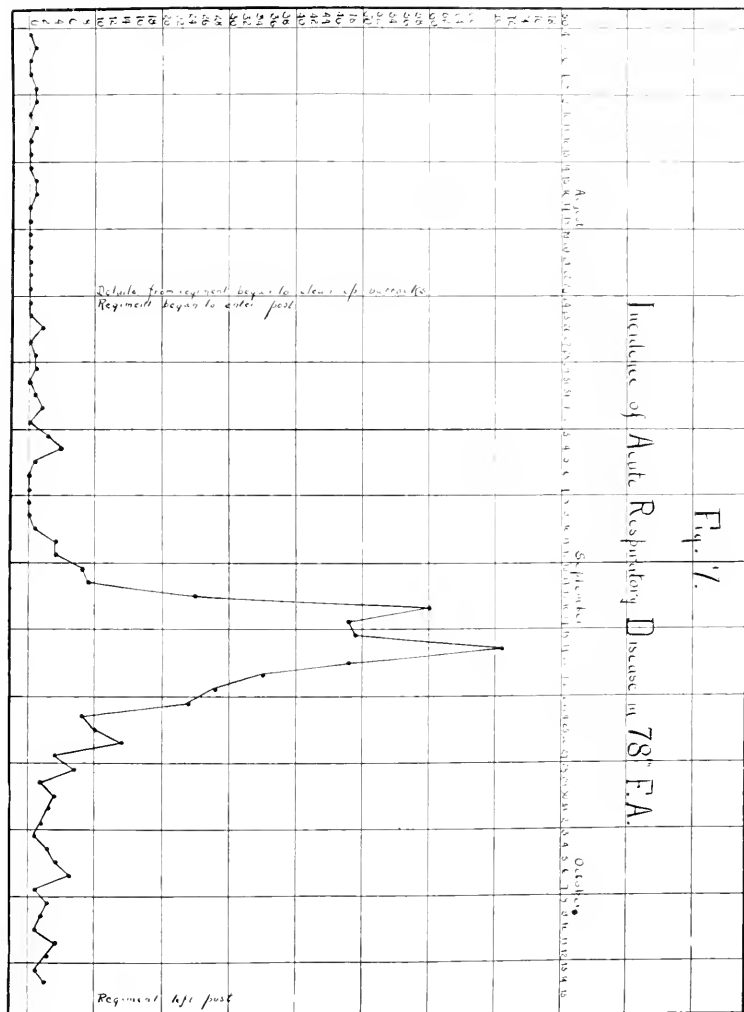
ing to the 6th Brigade and the remaining 181 in permanent post organizations.

In Figs. 5, 6 and 7 are illustrated curves which show the number of cases of respiratory disease admitted to the hospital daily from the three regiments of the 6th Brigade, before their entry into the post. These curves show that these regiments were almost free from cases of respiratory disease previous to moving into camp, and that influenza was not a serious menace until two weeks after their entry into the post.



In Fig. 8 is shown the daily incidence of respiratory disease in organizations barracked in the post during the period July 1st to October 21st. It shows that as successive brigades entered the post there was a progressive increase in the number of soldiers who developed influenza. The epidemic as it manifested itself in the first of the brigades was relatively mild, in the second brigade to enter the post it was more severe and in the third it was more severe than in the other two. Thus in the first brigade to enter the post the morbidity may be estimated at about 1.5 per cent, in the second at about 6.5 per cent, and in the third it reached the high figure of about 34 per cent.

It is seen from Table III that there was considerable variation in the extent to which different organizations were affected. The highest incidence in any one battery occurred in Battery C of the 3rd F. A. regiment and amounted to 141 cases, a morbidity of about 70 per cent. The least incidence in any one battery of the same brigade during the same period was that occurring



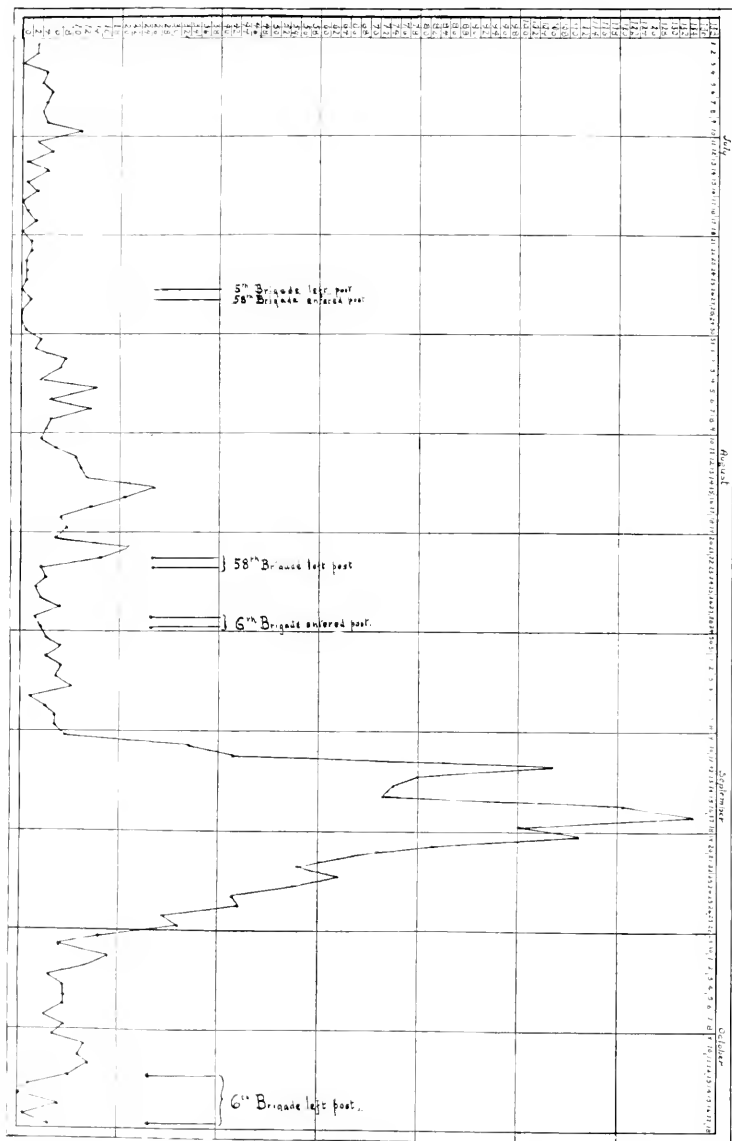


FIG. 8. Occurrence of Influenza in Organizations Barracked in the Post of A. P. O. No. 704.

in Battery F of the 3rd F. A., which had only 15 cases. There was a tendency for the disease to appear in an organization with what may be termed "explosive violence," a large number of men in a single battery coming down with the disease on the same day, when the battery had previously been almost free from the disease. The greatest number of admissions to hospital from any one battery in one day was 40.

It was impossible to prepare a spot map to show the barrack location of each case occurring during the third period of the epidemic for the reason that the units were frequently shifted about in barracks and this factor made it impossible to trace the individual cases. However, it is worthy of note that the two regiments of the 6th Brigade which suffered the most moved into barracks that had been occupied by the two regiments of the previous brigade in which influenza had been most prevalent. (See Fig. 4.)

Once the disease had become well established, it spread rapidly through the various commands and the epidemic ended apparently by burning itself out. The 6th Brigade began to vacate the post on Oct. 13th and the last regiment left on Oct. 21st. Although the date of the departure of the 6th Brigade marks the end of the epidemic of influenza in the post, it does not mark the end of the epidemic in the vicinity, for shortly after the epidemic of influenza in the 6th Brigade reached its height, influenza began to be epidemic among the troops of the 156th Artillery Brigade which at that time was billeted in surrounding towns.

This brigade arrived in the vicinity of the Post of A. P. O. 704, during the last week of August 1918, having come almost directly from the United States. It had had no influenza previous to its arrival in the nearby towns where it was to be billeted, but about three weeks after arrival in the billeting area influenza began to be epidemic in one of the regiments and within a week the other two regiments had become affected. These regiments were billeted in three separate towns, situated many miles apart, and yet there was not the difference of a week in the date of onset of the epidemic in the three regiments. The epidemic in the 156th Brigade had practically reached its end by the time the 6th Brigade was ready to leave the post, so that no objections to the entry of the former brigade into the post were raised by the medical authorities. The 156th Brigade began to enter the post Oct. 21st, and left on Nov. 8th. Only 28 cases of influenza developed in this organization during its stay in the post. The entire number of cases developing in this brigade during its stay in the neighborhood of LeValdahan amounted to 1048, a somewhat smaller number than that occurring in the 6th Brigade. The general characteristics of the outbreak in this brigade were the same as those in the 6th Brigade.

The total number of cases diagnosed as influenza, acute bronchitis, bronchopneumonia and lobar pneumonia, occurring in organizations barracked within the post of A. P. O. 704, A. E. F. or billeted in the vicinity, during the period July 1st to Nov. 8, 1918, amounted to 3162. By far the majority of these cases developed within the post and were admitted to the post hospital, Camp Hospital No. 12, although a few were cared for in regimental infirm-

aries as long as no complications developed. None of the permanent post organizations escaped infection, those most seriously affected being the camp hospital detachment, the 3rd Veterinary Hospital and the Quartermaster Corps detachment.

A number of cases of influenza developed pulmonary complications. It is difficult to be sure of the exact number of such cases, particularly during the earlier part of the epidemic. From Sept. 1st to Nov. 8th, when respiratory diseases were most prevalent, there were admitted to the Camp Hospital No. 12 or there developed within the hospital, 324 cases of bronchopneumonia or lobar pneumonia. This figure represents about 12 per cent of the total number of cases of respiratory infection that occurred in the vicinity of the post of A. P. O. during the same period. Of these 324 cases of pneumonia (broncho- and lobar) 151 died, giving a case mortality rate of 46 per cent for the lung complications. In addition to the cases with pulmonary complications there were a number of cases in which meningococcus meningitis developed during the period of convalescence from influenza.

#### D. DISCUSSION

The epidemic of influenza at LeValdahon offered an unusual opportunity for the collection of data which might throw light on the epidemiology of this disease. The exposure, in turn, of large bodies of men of approximately the same age and general physical condition to infection, and the fact that the previous history of these bodies as regards respiratory disease could be accurately ascertained for a period of time sufficiently long to permit of an estimate of the prevailing health of the troops as a whole, made this particular epidemic a natural experiment with unusually good controls.

Considerable interest attaches to the problem as to where and by what channels the various brigades acquired their infection. With regard to the first brigade to enter the post, the 5th F. A. Brigade, it is impossible to determine the source of the infection. The most probable source would seem to be the civilian population of Besancon, a city situated about thirty kilometers from the post and one to which the men were permitted to go on pass.

The source of infection in the case of the next brigade to enter the post, the 58th F. A. Brigade, seems fairly clear. The brigade undoubtedly acquired its infection as a result of moving into infected barracks, for the two regiments of the brigade which moved into the barracks in the post began each to have an epidemic of influenza three and five days respectively after moving into the post, although these regiments had previously had no cases of influenza, while the other regiment, which did not enter the post, had no epidemic at all. On account of the short interval elapsing between the entry of the brigade and the onset of the epidemic, as well as the fact that the epidemic in the two regiments began almost simultaneously it would seem most likely that the first soldiers to be affected acquired their infection as a result of living in infected barracks rather than from carrier or incipient cases among the post personnel. Had the infection been acquired through the latter channels one would have expected a longer interval before the onset of the epidemic, for there was not much opportunity for direct contact between the



permanent post personnel and the members of the visiting brigades. On the whole, then, the evidence would seem to point to the view that the 58th Brigade acquired its infection as a result of moving into barracks that had previously been inhabited by an infected body of troops. If this view is correct, it must be assumed that the more immediate source of infection was the dust that had accumulated on the floors and bedding of the barracks and had been contaminated with the oral secretions of the men, and also that the virus of influenza can retain its virulence for several days under such conditions.

The source of infection in the case of the next brigade is not quite clear. It is quite true that this brigade (the 6th) had little or no respiratory disease prior to its entry into the post, and the fact that a period of a fortnight intervened between the entry of this brigade into the post and the beginning of the epidemic indicates that the brigade was not infected at the time of entry nor was it on the verge of an epidemic at that time. It must therefore have acquired its infection in the post itself. The next question that arises is: Was the infection acquired as a result of moving into infected barracks, the actual source being infected dust left behind by the predecessors, or was it acquired from carriers or incipient cases among the post personnel? It is almost impossible to decide this question conclusively as the evidence is conflicting.

In favor of the view that the infection was acquired directly from the barracks is the fact that those regiments (3rd and 78th F. A.), which moved into the southwestern group of barracks suffered most, and these particular barracks were the ones that had been occupied by the regiments of the previous brigade which had shown the largest number of cases. There were proportionately twice as many cases in the southwestern group as in the southeastern group, which was occupied by the other regiment of the brigade, the 11th Field Artillery.

Against the view that the infection was acquired from the barracks is the fact that the southwestern group of barracks was cleaned fairly thoroughly before the troops moved in and also the fact that a considerable interval of time (a fortnight) elapsed between the entry of the troops and the onset of the epidemic. Had the 6th Brigade acquired its infection as a result of moving into infected barracks, one would have expected the epidemic to commence at an earlier date.

The possibility exists that the infection was acquired by those soldiers of the 6th Brigade who participated in the cleansing of the barracks and by this means the disease was transmitted to others. The preliminary part of the cleansing process, the handling of the bedding, was a dusty affair and the infection may well have been acquired in this manner although there is no positive evidence on this point. The fatigue parties that did the work were changed daily so that in the course of several days there were several different groups exposed.

Again, the infection may have been acquired from the permanent post personnel. There were always cases occurring in the post organizations and

it may have been that carriers or incipient cases in these organizations served as intermediaries of infection for the incoming brigade. On the whole this view seems to explain the source of infection in the 6th Brigade as well as any. It would fit in with the longer interval of time elapsing between entry into the post and onset of the epidemic observed in the case of this brigade.

The origin of the epidemic in the last brigade to enter the post, the 156th Brigade, is not of particular interest as the brigade underwent its epidemic while billeted in neighboring towns and entry into the post apparently did not alter the course of the epidemic in the brigade in any manner.

The fact that during the Summer and Autumn of 1918 three brigades of approximately the same strength entered the post of A. P. O. 704 and were quartered there in succession, and that each underwent an epidemic of influenza which was more severe than that sustained by the preceding brigade would indicate that the virus of the disease became progressively more virulent as succeeding groups of troops were attacked. It has long been known that the virulence of bacteria may be increased by successive passage through susceptible animals. The epidemic at LeValdahon would seem to offer an instance of increased virulence acquired by the virus of influenza as a result of successive human passage. This assumption would explain the almost step-like increases in severity of the epidemic as observed in different brigades.

Against such an explanation of the increased severity of the individual "brigade epidemic" it might be urged that, as each brigade became infected in turn and moved away it left behind a greater amount of infected material and as a result each incoming brigade received in turn a larger dose of infecting material. However, this argument can be met with the fact that the brigade which suffered the most, the 6th, moved into barracks which had been scrubbed and were as clean as it was possible to make them under the existing conditions, and also that the cleansing process instituted was one calculated to remove at least the greatest amount of such infectious material if it were present. In view of the fact that such cleansing measures were instituted before the brigade moved in, one would certainly not have expected in that brigade a more severe epidemic than that sustained by the preceding brigade had the virulence of the infecting agent and the resistance of the troops remained the same. The evidence is strong, therefore, that in this particular epidemic we have a fairly clear-cut demonstration of increased virulence acquired by the virus of influenza as a result of successive passage through human hosts.

The chief lesson to be learned from the epidemic, from a military standpoint, is the great danger of moving noninfected bodies of troops into barracks in which there have previously been organizations affected with respiratory diseases in epidemic form. This is by no means a new lesson. In the second place the experience at LeValdahon shows that not even cleansing measures as thorough as it is possible to make them under good campaign conditions will suffice to prevent infection entirely. It would seem that better results could be obtained by a complete avoidance of infected barracks.

rather than by trusting solely to the simple measures of cleansing that are possible and practicable when dealing with large bodies of troops.

#### E. SUMMARY

1. During the period July 1st to Mar. 1st, 1918, a respiratory infection diagnosed as influenza occurred in epidemic form among United States troops located in or around the post of A. P. O. 704, American Expeditionary Forces, France, situated at the French Artillery Camp LeValdahon.

2. More than 3,000 cases occurred in this epidemic.

3. Approximately 12 per cent of these cases developed pulmonary complications, with a resultant case mortality rate of approximately 46 per cent.

4. The epidemic manifested itself in a series of successive outbreaks as different artillery brigades, hitherto unexposed to the disease, were in turn exposed.

5. These successive outbreaks tended to be progressively more severe both in character and extent. Evidence has been brought forward to indicate that this fact was due to increasing virulence acquired by the infecting agent.

# LABORATORY METHODS

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## A NOTE ON THE EFFECT OF AMINO ACIDS ON THE GROWTH OF TUBERCLE BACILLI\*

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BY PETER MASUCCI, GLENOLDEN, PA.

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### I. INTRODUCTORY

THIS experiment was undertaken in response to complaints from the Tuberulin Laboratory that for several months the growth of tubercle bacilli on glycerine bouillon was very scanty. The same experience was reported by several laboratories throughout the country. Owing to the change in the peptone used, the peptone was held as the cause of the limited growth. It is a well-known fact that amino acids are essential in starting the growth of bacteria. They include elements of the much more complex bacterial protein. Amino acids, therefore, are the ultimate foodstuffs from which bacteria derive their essential requirement of nitrogen.

Experience had shown that a medium rich in amino acids influenced to a marked degree the growth of *B. diphtheriae*, although there was not as a rule a corresponding increase in toxin production. Since the tubercle bacillus is a very slow growing organism, it was thought possible that a glycerine bouillon enriched in amino acids would hasten the growth as well as increase it. Glycerine bouillon was prepared containing various amounts of amino acids, and the effect on the growth of the tubercle bacillus was carefully noted.

### II. EXPERIMENTAL

Glycerine bouillon was prepared according to standard procedures for bacteriologic purposes. The meat juice was prepared by the cold infusion method. Five hundred grams of ground beef were infused in 1000 c.c. of water overnight. The material was filtered and the filtrate made up to 1000 c.c. The meat juice was then divided into two parts, to one (A), was added 2 per cent "Difco" peptone; to the other (B), 1.7 per cent "Difco" peptone plus 0.3 per cent "Aminoids" (Arlington Chemical Co.). The reaction of both after the addition of 5 per cent glycerine, was adjusted as nearly as possible to the same pH. After sterilization a sample flask from each lot was analyzed. The solids, ash, and total nitrogen were determined by the methods of the A. O. A. C. Special precautions were taken in determining the solids to avoid the volatilization of glycerine. The bouillon was heated in a vacuum oven to a constant weight at 75 °C. for 48 hours. Total phosphorus was determined

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\*From the Mulford Biological Laboratories, Glenolden, Pa.

by Sato's<sup>1</sup> colorimetric method. Protein nitrogen was found by saturating the bouillon with  $ZnSO_4$  in a slightly acid solution, and a Kjeldahl determination was made on the precipitate. The amino acids were determined by the Van Slyke method.<sup>2</sup> Both lots of bouillon were adjusted to PH 7.5 electrometrically. The results of the analyses of the two lots are given in the following table in percentages:

TABLE I

	Solids	Ash	P <sub>2</sub> O <sub>5</sub>	N	N ppt. by $ZnSO_4$	N as amino acids
A.	9.22	1.05	0.116	0.348	0.119	0.098
B.	9.19	1.00	0.176	0.369	0.098	0.130

It will be seen by the analyses that Lot B is much richer in amino acids and total phosphorus than Lot A. Lot B has 31 per cent more amino acids and 50 per cent more phosphorus than Lot A. The larger amount of phosphorus in Lot B is perhaps as instrumental in influencing the growth of the



FIG. 1.

tubercle bacillus as the amino acids. This is made more evident when we consider the chemical constituents of the tubercle bacillus. This organism is rich in lipoids and especially in nucleo-proteins and lecithin proteins, both substances containing phosphorus in their molecule. It may be readily seen, therefore, that phosphorus plays an important part in their growth. Both lots of bouillon were planted with *B. tuberculosis*, human and bovine types, and incubated at 37° C. At the end of ten days, growth on Lot A was very scanty. Pellicle formation had not yet started. Lot B containing the aminoids, showed profuse growth with a complete surface pellicle. The amount of growth at this period was far greater than that of Lot A after six weeks of incubation. The bottle to the left (Fig. 1) shows the growth of Lot B and the one on the right that of Lot A. The difference in growth is very marked.

Several lots of glycerine bouillon were made containing 0.1 per cent to 1 per cent aminoids. The best growth seems to take place in bouillon containing 0.3 to 0.5 per cent aminoids. The bouillon now used in the Tuberculin Laboratory contains 1.7 per cent "Difco" peptone and 0.3 per cent aminoids. The results obtained are very satisfactory.

The function of glycerine in glycerine bouillon is not exactly known. Some writers claim that the tubercle bacillus does not utilize glycerine. However that may be, the osmotic pressure of Lot A and B was determined by the cryoscopic method. The results are given in the following table.

TABLE II

	Delta	Osmotic Pressure in Atmospheres
A.	2.610°	31.33
B.	2.580°	30.98

Ordinary plain bouillon has a freezing point depression of 0.6° to 0.9° C. or an osmotic pressure of 7.23 to 10.84 atmospheres. Whether glycerine plays any other function or not, one thing is certain, it increases the osmotic pressure of the bouillon threefold.

## SUMMARY

Glycerine bouillon containing 1.7 per cent "Difco" peptone and 0.3 per cent aminoids has about 30 per cent more amino acids and 50 per cent more phosphorus than bouillon made with 2 per cent "Difco" peptone alone. The amino acids hasten the growth of *B. tuberculosis* by furnishing the necessary form of nitrogenous matter for the building of the more complex bacterial protein. The increased amount of phosphorus probably increases the quantity of growth as that latter element is an important constituent of nucleic and lecithin protein, substances found abundantly in the tubercle bacilli.

Whatever the function of glycerine is in glycerine bouillon, it is certain that the osmotic pressure of glycerine bouillon is three times greater than plain bouillon.

## REFERENCES

- <sup>1</sup>Sato, A.: Jour. Biol. Chem., 1918, xxxv, p. 473.  
<sup>2</sup>Van Slyke: Jour. Biol. Chem., 1912, xii, p. 277.

## "VARIATIONS IN THE WASSERMANN REACTION"—A REPLY\*

By ROBERT A. KILDUFFE, A.M., M.D., PITTSBURGH, PA.

IN the July, 1920, number of this Journal appeared a paper by Wilson† which, as tending, perhaps, to confuse the clinician depending upon the Wassermann test as a source of information, seems to invite some discussion.

Briefly, the paper emphasizes the following points:

1. That a cholesterinized antigen is more sensitive than an acetone-insoluble extract of beef heart.
2. That a cholesterinized extract may give a slight reaction with a normal serum.
3. That a positive Wassermann may be had in leprosy, frambesia, "hepatic disease," and scleroderma.
4. That blood taken after anesthesia may give a false positive reaction.

\*From the Laboratories of The Pittsburgh Hospital, Pittsburgh, Pa.

†Wilson: Jour. Lab. and Clin. Med., July, 1920, v, No. 10, 670.

5. That serums should be collected in sterile tubes and examined as soon after collection as possible.

6. That "we have repeatedly demonstrated than anticomplementary substances develop in serums if kept for a considerable time, which not only inhibit hemolysis in antigen and control tubes, but certain substances are formed, presumably by bacterial growth or action on some substances in the blood serum, creating an inhibition of hemolysis in the antigen tubes alone, causing, perhaps, a negative serum to become positive."

7. That care should be taken in interpreting the significance of "weakly positive up to two-plus reactions" in cases giving a negative history and without symptoms of syphilis.

8. That reactions of varying degree may be obtained with different antigens and the same serum.

Many of the above statements are, of course, merely repetitions of facts well known to serologists in general and embody nothing new.

That cholesterinized extracts must be controlled by less sensitive antigens is so well realized that it is safe to say that no competent serologist, now-a-days, would attempt a complement-fixation test with less than two antigens—a cholesterinized extract and an acetone extract—the writer using, in addition, a plain alcoholic extract of either normal beef or human heart, or luetie liver.

Moreover, no antigen of any kind should be used whose full anticomplementary and antigenic dose is not known, and which has not been tested, along with well-known and tested antigens, in at least from twenty to fifty tests to ascertain that its reactions are consistent and reliable. In addition, knowing that antigens may rapidly and suddenly deteriorate, all antigens should be re-titrated at monthly intervals.

If these conditions are fulfilled, fewer inconsistent and inconstant reactions will be obtained.

To one accustomed to using a triple antigen battery, the varying strength of reactions obtained with varying antigens is well known. It has not been my experience, however, that the untreated, four-plus case gives anything but four-plus with all three. In the treated case, the varying reactions with varying antigens is well-illustrated and can be utilized as a means of following the efficacy of the treatment, as the reaction first disappears in the plain extracts, then in the acetone extract, and lastly in the cholesterinized extract which may disappear very slowly.

In regard to the nonspecific, positive reactions obtained with cholesterinized extracts: while this may be taken as the attitude of serologists in general, time alone will show if this error is as marked as it is said to be, and, in any event, the error is controlled by the use of multiple, less sensitive antigens.

The question resolves itself, as has been stated time and time again, into one of the *interpretation* of the test, and not the test alone, a matter which I have discussed elsewhere.\*

In the unknown case, submitted for diagnosis, one would hesitate, in the presence of a negative reaction with all but the cholesterinized extract, to

\*Kilduffe: The Practical Value and Utilization of the Wassermann Test in General Practice, Arch. Diagnosis, Jan., 1920.

forthwith diagnose syphilis. Experience, however, would indicate the advisability of a repetition of the test after a short course of antiluetic treatment, in other words, a "provocative Wassermann."

With the same reaction in a patient under treatment, or where the history is to be had, such a reaction can justly be interpreted as indicating the necessity for further treatment.

In the weakly positive case without history or symptoms, it is a simple matter to repeat the test with another triple antigen battery, or, better still, to do a provocative Wassermann which is likely to be plainly positive. That a positive Wassermann may occur, in the absence of syphilitic infection, in leprosy and yaws is well known. Yaws has, for its etiologic agent a spirocheta, (*Spirochetæ pertenuis*) which is almost indistinguishable, morphologically, from *Treponema pallidum*—a fact which may account for the occurrence of the reaction. In any event, neither leprosy nor yaws should be mistaken for syphilis, so that this fact does not vitiate the reliability of the Wassermann.

That a positive Wassermann, in the absence of syphilis, may also be had in "hepatic diseases" in general, is, perhaps, not so universally admitted; and that positive reactions obtained in "hepatic diseases" are always obtained in the absence of syphilis, is, perhaps, debatable.

The question of positive reactions in scleroderma cannot be given a marked importance as affecting the reliability of the test, for, occasionally, syphilis and scleroderma are co-existent and, when this is not the case, the condition is one not easily confused with syphilis. The cause of scleroderma being entirely unknown, it is not beyond the bounds of probability that the positive reactions may arise from the same combination of circumstances responsible for them in frambesia—the presence of a spirocheta closely related to the *Treponema pallidum*.

The knowledge of the necessity of sterile glassware and of collecting and keeping serums under aseptic conditions goes back to the days of the first description of the test, and the necessity for avoiding bacterial contamination is, likewise, well known.

It is with the last statement in the paper that this reply is principally concerned. If it is true that "bacterial contamination and bacterial action on some substance in the blood serum," will create "an inhibition of hemolysis in the antigen tubes alone, thus causing a negative serum, perhaps, to become positive"—this statement, though justly qualified by "perhaps," should arouse much interest. Certainly, the exact methods whereby such a conclusion could be reached should be known, so that the observation may be repeated and confirmed by others.

Personally, the writer feels that a complement-fixation test properly performed, with antigens whose anticomplementary and antigenic range is definitely known, and in the presence of adequate controls, *when properly interpreted*, constitutes a safe and reliable guide to the diagnosis of syphilitic infection.

The absence of a history is not always to be relied upon; the absence of symptoms readily detectable, is a phenomenon of frequent occurrence in this



protean disease; and the conditions other than syphilis in which a positive fixation may occur are too morphologically distinct to be a justifiable cause for confusion.

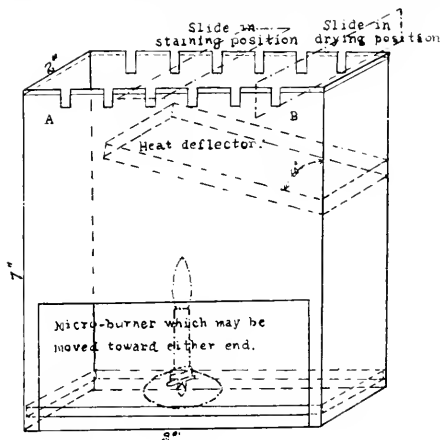
That the reaction has its limits of delicacy is, of course, well known, and it is to be hoped that there are few clinicians, and still fewer serologists who would place absolute dependence upon a single negative Wassermann as indicating absolute freedom from syphilitic infection; conversely, the occurrence of a positive below the four-plus variety, is to be always intelligently interpreted in terms of its relation to the patient and his condition.

## APPARATUS FOR STAINING AND DRYING SLIDES

BY FREDERICK H. LAMB, M.D., DAVENPORT, IOWA

THE accompanying diagram represents an apparatus for staining and drying microscopic slides, which I have used in laboratory work for five or six years. It is easily made by any copper-smith, inexpensive and has proved to be very satisfactory.

The apparatus consists simply of an oblong box, made of sheet copper, preferably, although galvanized sheet iron will serve the purpose and is less expensive. The top of the box as it stands in the diagram is open. Along



each side and directly opposite to each other, there are small slots  $1\frac{1}{2}$ " wide and  $\frac{1}{2}$ " deep spaced 1" apart, in which slides may be placed to drain and dry. The bottom of the box consists of a piece of sheet copper with flanged edges and placed as shown in the diagram. This serves as a stand or base for a micro-burner. One side of the box is cut out as shown, so that the burner may be moved from one end to the other, depending on the temperature which is desired under the slides. About half way between the bottom

and top of the box there is a piece of copper sheeting with edges flanged and inclined as shown in the sketch. This acts as a heat deflector. It does not extend across the entire width of the box, but stops short of one end at about 1½ inches, so that if necessary heat from the burner may pass directly up to the slide. The over-all dimensions are: 8" wide, 7" high and 2" deep. The width may be increased an inch or two to accommodate more slides if so desired.

The apparatus is especially useful in staining with carbol-fuchsin for tubercle bacilli. The slide may be placed in the position "A" and with the microburner in position "X," there will be just enough heat to cause the stain to steam. With a slide in position "B" the temperature is about 10° C, above room temperature.

Aside from its direct usefulness, the apparatus affords a safe and clean way of handling infectious material.

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## BLOOD CHANGES IN A CASE OF HEMOPHILIA AFTER TRANSFUSION\*

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BY HAROLD A. BULGER, M.D., BOSTON, MASS.

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ALTHOUGH the use of Blood serum and blood transfusion in hemorrhagic diseases is not an uncommon procedure, there are few instances on record in which even the effect on the coagulation time has been described. It is of interest, therefore, to report the following study of the changes in the factors of coagulation in a case of hemophilia.

It is well recognized that the results of transfusion in hemophilia are temporarily beneficial even if they are not permanent. How lasting the results are is not definitely known. Information on this question would be of value as indicating whether the degree of change is great enough to allow operative procedure.

Minot and Lee<sup>1</sup> report a case with coagulation time of 150 minutes. After transfusion of 600 c.c. of human blood (method not stated) the coagulation time was normal, but in three days it was 60 minutes and in five days 100 minutes. Addis<sup>2</sup> reports two cases. In one, injected intravenously with 15 c.c. of human serum, the coagulation time was 62 minutes before and 24 minutes after injection. After twelve days it had risen to 127 minutes but in three weeks had fallen to 86 minutes. The second case was transfused with 300 c.c. of phosphated blood. Before transfusion the coagulation time was 245 minutes; after transfusion 24 minutes. After twenty-five days the coagulation time was 200 minutes and at that time 8 c.c. of human serum injected intravenously brought it down to 38 minutes.

A boy (R. E. W., Med. No. 11486), age 14 years, with a typical family history of hemophilia, had had attacks of swelling in the joints and at one time

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\*From the Medical Clinic of the Peter Bent Brigham Hospital, Boston, Mass.

This is study No. 8 of a series of studies on the physiology and pathology of the blood from Harvard Medical School and allied hospitals.

very serious bleeding after the extraction of a tooth. For the past five years he had repeated attacks of hematuria averaging about two weeks in duration. Three weeks before admission, following whooping cough, blood appeared in the urine again, and the night before admission his left knee became greatly swollen. During his stay in the hospital the hematuria gradually disappeared but the other knee became swollen and later both elbows became involved. He was transfused by Dr. Cunningham with 300 c.c. of his mother's blood by the citrate method, and following this the swelling gradually subsided. Eleven days after transfusion his right elbow and ankle both showed fluctuant swelling again but they rapidly subsided. Hematuria did not recur.

Just before transfusion and at various intervals following, blood was obtained by veni-puncture in a glass syringe rinsed in salt solution. Part of the blood was used to determine the coagulation time while the remainder was oxalated and the oxalated plasma separated. On most occasions a normal control

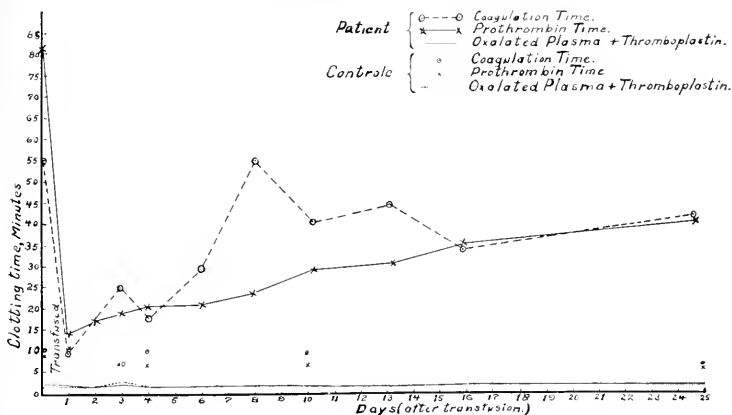


Fig. 1.

specimen was obtained in a like manner. With each specimen of oxalated plasma the "so-called" prothrombin time was determined by the method described by Howell and also the effect of adding thromboplastin solution on the coagulation of the oxalated plasma. The changes are shown by the accompanying graphs.

#### COMMENT

It will be noted that the day following transfusion the blood was practically the same as the control, but that the coagulation time and prothrombin time gradually lengthened. One month later they were still less than before transfusion. There was no marked change in the blood found to correspond to the slight bleeding into the joints found on the eleventh day.

The effect of thromboplastin on the clotting of the oxalated plasma is of interest in relation to the cause of hemophilia. It has been suggested that hemophilia is due to insufficient prothrombin in the blood, but in this case the addition of thromboplastin to the oxalated plasma caused it to coagulate

as well as the normal control. This suggests that there was sufficient prothrombin and that thromboplastin was lacking.

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<sup>1</sup>Minot and Lee: Arch. Int. Med., 1916, xviii, 1.

<sup>2</sup>Addis: Proc. Soc. Exper. Biol. and Med., 1916, xiv, 19.

## A SIMPLE METHOD OF ISOLATING BACTERIA FROM PATHOLOGIC MATERIAL\*

BY H. C. KLIX, DETROIT, MICH.

THE object of this paper is to present a simple and rapid method for the isolation of bacteria from pathologic material. It has been used by the writer for a number of years in the routine examination of mixed cultures, pus, sputum, and other material submitted for the preparation of autogenous vaccines. It offers a number of advantages over the ordinary plating method.

The use of Petri dishes is not only cumbersome, but is unadapted to certain culture media, particularly those containing unheated body fluids. The exposure incident to handling plates also increases the danger of contamination. The following method overcomes these disadvantages, is simple of execution, and can be satisfactorily applied to practically all bacteriologic examinations.

## EQUIPMENT

A few tubes of suitable culture media, usually ascites agar slants, a small tube of bouillon, and a platinum loop.

## TECHNIC

One-fourth c.c. of bouillon or water of condensation is pipetted into each of four tubes of solid media and the tubes numbered I, II, III, and IV. A small amount of pathologic material is transferred to Tube I and mixed by means of platinum wire with liquid (bouillon or water of condensation). Two or three loopfuls of the bouillon in Tube I are transferred to Tube II and mixed as before. Two loopfuls of bouillon from Tube II are transferred to Tube III. One loopful from Tube III is transferred to Tube IV.

Tubes are shaken to break up any clumps of original material or bacteria, and bouillon is allowed to run over the entire surface of the slant by properly inclining the tube. Tubes are placed in rack in upright position and transferred to incubator. After 24 hours' incubation isolated colonies are picked up by means of platinum wire and transferred to individual tubes of culture media.

Somewhere in the series nicely isolated colonies will be found which can be readily picked up.

The chief advantage of this method aside from its simplicity is its adaptability to any kind of culture media.

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## EDITORIALS

### *Influenza and Tuberculosis*

FOLLOWING the 1918 and 1920 epidemics of influenza, there has arisen in the literature some controversy regarding the effect, if any, of influenza on tuberculous individuals. This has centered particularly on the question whether tuberculosis produces some degree of immunity to influenza, and whether the latter on the other hand predisposes either to the lighting up of a latent tuberculosis, or to a new infection with the tubercle bacillus. Keen observers in the field of tuberculosis who have had apparently equal opportunities to study the effects of the pandemic differ radically in their conclusions.

After the 1889-1893 epidemics, Leichtenstern recorded that the mortality tables of all countries agree in showing considerable rise in the mortality from pulmonary tuberculosis in influenza periods. The clinicians of that time made the frequent observation that the course of tuberculosis in the lungs is markedly and unfavorably influenced by grip and its pneumonic complications. Latent quiescent cases often became active, and healed and healing foci broke out anew. Afebrile cases were changed to the hectic type and frequently hemoptysis was induced. In London, during the height of

the 1889 epidemic, the weekly death reports from phthisis rose to double the average. The increase in death rate during the epidemic period was not limited entirely to tuberculosis, but there was almost a doubling of deaths due to all acute respiratory infections. After the cessation of the epidemic, however, there was some decrease in the general mortality, as well as in the mortality from respiratory infections. This was especially true of deaths from pulmonary tuberculosis, which decreased to such an extent that the total mortality rate for the year for this disease was little greater than for preceding years.

Similar observations have been made following the 1918 pandemic. Jordan remarks that in New York City in 1918 during the two weeks of maximum epidemic mortality, the deaths reported from pulmonary tuberculosis numbered 430, as compared with 264 for the corresponding weeks of 1917. Vaughan and Palmer found that the deaths from tuberculosis in the army were higher in the autumn of 1918 than in the two previous four months' periods, the death rate rising from 18 per 100,000 during the summer to 46 per 100,000 in the autumn. The rate for the same time of the preceding year had been 15 per 100,000. They assume that the most plausible explanation for this increase in deaths is that dormant and incipient cases introduced into the army during the preceding year had accumulated and possibly were hastened into the acute stage, both by the duties of camp life, and the prevalence of the epidemic of grip and pneumonia. Quite naturally there have been from the time of the first assembling of troops an accumulation of tuberculous individuals, inasmuch as such men were not discharged, but were kept in the army and under Government control and supervision. Sir Arthur Newsholme in reviewing the relationship between influenza and tuberculosis in England remarked that so far as the national statistics in that country are concerned, every year showing an extensive death rate from the former has been followed by a year in which the death rate from the latter has been excessive. He concludes that many deaths from tuberculosis are undoubtedly hastened during an influenza epidemic. Abbott wrote of the epidemic of 1889 in Massachusetts that the chief diseases which followed in its train and were intimately associated with it were bronchitis and pneumonia, and that phthisis when already existing in the victim of the attack was undoubtedly aggravated and in many cases a fatal termination was hastened. Baldwin says that influenza is a frequent and important agent in bringing latent tuberculosis to life. "Allowing for mistakes in diagnosis in which the presence of tuberculosis is overlooked, influenza must be classed as an important exciting cause, if not a true predisposition."

In frank opposition to the foregoing authorities, Fishberg claims that influenza has had no effect whatever on the course of tuberculosis. He says that a large proportion of tuberculous patients under treatment in New York City in 1918-1919 contracted the disease and not a single one succumbed. This appears as rather an inclusive statement. He goes on to say that some were in the far advanced stages of the disease, with large cavities in the lungs, and yet they passed through the acute symptoms and recovered, the tuberculous process then pursuing its course as if no complicating disease

had affected them. He believes that the prognosis was if anything better in those who suffered from tuberculosis or any other chronic pulmonary disease, such as asthma, bronchitis, emphysema, bronchiectasis, than in those in whom the lungs and bronchi had been apparently in healthy condition. Fishberg observes that, instead of lighting up the tuberculosis, the influenza runs a milder course than when attacking healthy persons, and the old lung lesion remains in about the same condition as could be expected if no complicating process had attacked the patient. He says that authors who have asserted the contrary have based their arguments mainly on the fact that many tuberculous patients date the onset of their tuberculosis as concurrent with an attack of influenza; that many patients suffering from phthisis state that ever since an intercurrent attack of influenza the symptoms of tuberculosis have become more pronounced; that the Pfeiffer bacillus has been found quite frequently in the sputum of tuberculous patients, especially that derived from pulmonary cavities; and that in some countries it has been noted that during and soon after an epidemic of influenza the mortality from tuberculosis was increased.

He believes that many of the conditions diagnosed as influenza have been no more than ordinary colds and that the average patient will call any upper respiratory tract infection grip during or around the time of an epidemic. He further believes that a misdiagnosis of tuberculosis is frequently made in influenza convalescents who show some signs of moisture in their lungs which does not clear up for some time, causing doubt in the mind of the examiner but which is not truly tuberculous in origin. Fishberg cites P. J. Murphy, Hawes, Armstrong, McRae, and Dickinson, as well as Geiber and Schlesinger in Vienna, and Riekmann and Ladeck in Germany, as having observed the same phenomenon of relative insusceptibility of tuberculous patients and failure of influenza to hasten the progress of tuberculosis. He also calls attention to the low incidence of influenza in tuberculosis sanatoriums, but apparently compares this incidence with the incidence for the public at large, and not with that in similar institutions devoted to the care of invalids with diseases other than tuberculosis, or with other institutions in general.

Amberson and Peters, as well as Minor, take sharp exception to the statement of Fishberg and the former have collected the evidence against Fishberg's view. They first point out that a comparison of the incidence of 5.4 per cent among hospitalized tuberculous patients at Chicago cannot be compared with a much higher incidence of the epidemic in the various military camps. As Heiser has pointed out, the mere quartering of men in barracks seems to have a tendency to increase the risk from acute respiratory diseases. Furthermore, the incidence at some sanatoria was low, while at others it was high, nearly as high as for the community at large. In Hawes' report of the epidemic among the Massachusetts sanatoria, Lakeville had escaped entirely, while Rutland which consisted chiefly of ambulatory cases less easily controlled, had an influenza incidence of 18.3 per cent among the patients, and 21.3 per cent among the employees. At Montefiore Home, the proportion of tuberculous patients and employees contracting the infection was practically the same as among the nontuberculous employees, and about

the same percentage of both groups developed evidence of broncho-pneumonia.

Still another fallacy in the comparison of incidence in institutions and the like is proved by the work done by Jordan, Reed and Fink, who found that in the various Chicago telephone exchanges the attack rate varied from 5 per cent to 27 per cent, although the working conditions were approximately the same. The attack rate in one section of the students army training corps in Chicago was 3.9 per cent, while in another section particularly exposed to infection it was 39.8 per cent. Similarly Frost found the incidence in Louisville, Kentucky, to be 15 per cent, and in San Antonio, Texas, 53.3 per cent. All of these figures show the difficulty of comparing rates for various institutions and various groups of individuals. Although Fishberg quoted Rickmann in support of his contention that influenza has no effect whatever upon tuberculosis, Amberson and Peters used his work in support of their contention, and call attention to the fact that in 30 out of 40 tuberculous persons reported by him who had contracted the grip, the attack did not produce any aggravation of the lung condition. Presumably it did in the other ten. If even 25 per cent of tuberculous patients who contract influenza have their pulmonary condition aggravated, this should be regarded as a notable number. According to Stivelman, 11.4 per cent of tuberculous influenza cases died at Montefiore Home. In a survey of convalescents from the Loomis Sanatorium, Amberson and Peters found that 70 had contracted influenza, or 5.7 per cent of the number surveyed, and that 11.4 per cent of these had had relapses of their pulmonary condition, apparently due to the acute disease, while 22.9 per cent had died from the intercurrent infection. Two and eight-tenths per cent were deaths due to tuberculosis after convalescence from the influenza. Tubercle bacilli have been found in the sputa of convalescent grip patients, whose sputa had previously been negative, by Amberson and Peters, as well as by Berghoff, at Camp Grant. The latter found that 50 per cent of his cases showed a reactivation and a positive sputum after an attack of influenza.

Amberson and Peters agree with Fishberg in the observation that there has been no increase in the general mortality from tuberculosis within the recent months, and suggest as an explanation the possibility that during the epidemic enough of the old cases were carried off to account for a temporary lull until new cases developed, or others had time to reach later stages of the disease. As we have previously remarked, Leichtenstern observed this same phenomenon following the 1889-1890 epidemic.

The state of our knowledge of influenza and tuberculosis is considerably clouded by divergent opinions such as those quoted above. To further complicate the picture, there are other authors who assume a middle ground and contend that there is some truth in both lines of contention. Thus, Amelung believes that the morbidity among patients with pulmonary tuberculosis is slight, and that the grip takes a milder course in such patients than in the nontuberculous unless the disease is far advanced, but that pulmonary tuberculosis may and sometimes does follow the disease in patients whose lungs



were previously sound, and that in the last-mentioned cases the prognosis is relatively bad. Peek finds that in some tuberculous patients the disease has been aggravated, but in the majority the intercurrent influenza did not appear to have been the causative factor in the acute exacerbation of the tuberculosis.

Debré and Jacquet have reviewed the European literature on the subject pro and con, and though they admit that there are exceptions, as at l'hôpital Tenon, where, in a barracks reserved entirely for female tuberculosis patients there was a veritable epidemic of grip, 29 per cent of the 28 being attacked in a few days; and at the sanatorium de La Tronche, where 83 per cent took ill between the 25th of September and the 20th of October; that as a rule tuberculous individuals are less heavily attacked by the influenza than are the nontuberculous. As they suggest, the first explanation that comes to mind is that the tuberculous are isolated in the hospitals where general hygienic conditions are good, but we have all seen other institutions, hospitals, etc., in which the inmates were not spared as they were in tuberculosis hospitals. Furthermore, in certain sanatoria, such as the sanatorium of the Côte Saint-André, and Bligny, and several German sanatoria, the proportion of tuberculous individuals attacked was very much less than that of the professional attendants, the physicians and nurses. Again, where cases have occurred in these hospitals, and little precaution was taken to prevent its spread, very few other individuals took sick. Finally, many have noted the infrequency of the disease even in those tuberculous individuals who were living at home. It has been suggested that rest in bed from the beginning of the attack explained the mildness, or that the immunity resulting from the infection with pneumococcus, streptococcus, etc., in tuberculous individuals explained the absence of pulmonary complications. Marfan, who observed this same phenomenon in 1890, suggested that it might be due to a refractory state of the tubercle bacillus against the virus of influenza. Debré and Jacquet conclude that none of these explanations is satisfactory.

Having concluded that tuberculosis does protect in some measure against influenza, Debré and Jacquet next discuss whether the latter has increased the severity of tuberculosis in the subjects who were already tuberculous. They review the literature and make their conclusions, not from statistical records, but from general observations. They consider first those cases of phthisis which are open cases when attacked, and second, latent tuberculosis. Their conclusion concerning the first group is that influenza does not have any effect on the rapidity of evolution of the tuberculous process, except in very rare instances, such as an occasional case of miliary tuberculosis following grip. As regards latent tuberculosis, however, they do believe that the intercurrent acute infection does cause in many cases a lighting up of a previously entirely dormant tuberculosis. It seems rather difficult to reconcile the two ideas. If one type of tuberculous individual is rendered more susceptible to the ravages of consumption, it would seem reasonable to expect that all types would be so affected.

The greatest difficulty in reaching a conclusion regarding the effects of

influenza on tuberculosis, and vice versa, is due to the fact that the individuals studied are in all stages of the disease, and that each individual reacts differently and in his own way. Opinions have been based chiefly on clinical observations, and not on statistical study of large series of cases, while from the nature of the conditions, even statistical studies would not be without great fallacy.

Armstrong, who has made as nearly a complete statistical study as is possible under the circumstances found that in Framingham, Massachusetts, 16 per cent of the entire population was affected with influenza, but only 4 per cent of the tuberculous group in the community was so affected. Most of these latter were of the arrested type and were going about the community taking their part in industry and exposed to the same degree of contact as was the case with the normal population. The fatality rate was equally in contrast. Armstrong concluded that there appeared to be a relative degree of protection for the highly tubercularized. If we accept these figures at their face value we must conclude then either that tuberculosis offers some degree of protection against acute influenzal affection, or, that the tuberculous of Framingham have been so well trained in sanitation and personal hygiene, as a result of the Framingham demonstration, that they have been able to protect themselves against the grip. In the latter case we must look upon the result as a successful demonstration of the principles of preventive medicine. Certainly this did play a part, to the extent at least that individuals knowing themselves to be infected with tuberculosis, and knowing themselves to be in the presence of a pandemic, became more wary of crowd contact, and in case they did become ill, they undoubtedly went to bed at the earliest opportunity.

If, on the other hand, this is a true demonstration of relative immunity in a chronically infected individual, the explanation must be sought elsewhere. Does a chronic respiratory infection confer a relative degree of immunity to an acute respiratory disease? Do the germs already on the premises exert, so to speak, "squatters' rights?" Are we observing an example of non-specific immunity due to local preceding infection? Still another factor must play an important role, the factor of race stock. The excess of tuberculosis in negroes, for instance, over that in whites, is in some localities double or treble, while various observers, as Frost, Brewer, and Frankel and Dublin, report that the influenza incidence and mortality among negroes was decidedly less than that among the whites. Winslow and Rogers found that in Connecticut the proportion of influenza-pneumonia deaths is lower than would be expected among persons of native Irish, English and German stock, and higher than was to be expected among Russian, Austrian, Canadian and Polish stock, while it was enormously high among the Italian. Italians are notably insusceptible to tuberculosis, while the Irish are much more prone to infection with the disease. For instance, in Framingham, where the tuberculosis incidence rate for the entire population was 2.16 per cent, the rate in the Italian race stock was .58 per cent, and in the Irish 4.80 per cent. In Framingham there was about four times as much influenza among the Italians as among the Irish. Is this apparent insusceptibility of certain race stocks an inherent condition, or is it dependent chiefly on differences in living

conditions and in age prevalence in the different races? Probably it is chiefly the former. Frost, for instance, found that among the negroes the incidence of influenza was lower even though the living conditions were much poorer than those among the whites.

Armstrong's survey has also thrown some light on the effect of the influenza on previously tubercularized individuals. In a survey of 700 individuals who had had the acute disease there were ten arrested cases of tuberculosis, or 1.4 per cent. All these had been known to be arrested cases previous to the epidemic, and in none of them did the disease appear to have been actively and permanently lighted up. Some had manifested a slight activity, but all seemed to be on the way to a rearrest of the disease. On the other hand, 13 cases, or a 2 per cent of the 700, were found to have active tuberculosis which had hitherto been undiagnosed, and an additional 8 cases, with indefinite bronchopulmonary signs, were designated as incipient tuberculosis cases. This is to be contrasted with an incidence of active tuberculosis in the preepidemic examination of approximately 1 per cent. These figures would indicate an increase in tuberculosis incidence. How may this be explained?

It has long been known that individuals with measles will not react to tuberculin tests, even though they have been positive before developing the measles, and though they will become positive again after recovery. The same may be said of vaccination. Individuals vaccinated against smallpox, who have measles, and are during their illness revaccinated, will not show an immediate reaction. The test will remain entirely negative, while after recovery, the immediate reaction may be obtained. Normally, it will appear in 95 per cent of cases, while among those with measles the phenomenon remains absent in 90 per cent. The same may be said of certain other acute illnesses, particularly scarlet fever. The phenomenon has been variously explained. Von Pirquet, who was the first to observe it in measles, believed that the acute disease created a temporary inability to produce antibodies, and therefore designated the condition by the name *anergie*. The same phenomenon of *anergie* has been found recently to hold in the case of influenza. Debré and Jacquet, Lereboullet, Bloomfield and Mateer, as well as Berliner and Schiffer, have brought abundant evidence to this effect following the 1918 pandemic. It has also been shown by Cayrel and others that there is a diminution of typhoid agglutinins in the serum of influenza patients vaccinated against typhoid. The agglutinin titer again increases after recovery. It is true that agglutinin titer is not a measure of immunity, but it is frequently used as such and serves to give us some information on the subject. If, then, influenza is an *anergie* disease, a "*maladie anergisante*," we have a theoretical explanation of the increase in severity of tuberculosis following the acute infection. We have long known that measles predisposes to tuberculosis. We have recently been thoroughly convinced that influenza lessens resistance to secondary infection with streptococcus, pneumococcus, and other respiratory tract organisms. Shall the tubercle bacillus be added to this list? During the 1918 epidemic we saw men in the army camps who passed through an attack of influenza-pneumonia and died within a few weeks from tuberculous pneumonia or miliary tuberculosis. These men had previously been so free

from signs of their tuberculosis as to be accepted for military service as healthy individuals. The number of these cases was small, to be sure, but sufficiently large to convince us that there do exist instances in which tuberculosis is tremendously fired by an intercurrent influenza.

If we may judge merely by the balance of evidence and risk any conclusions from such conflicting testimony, we may sum up as follows:

1. Great variation in the interaction of tuberculosis and influenza must be expected, because of the many stages at which the tuberculous may be attacked, because of the altered mode of living of known consumptives, and because of the protected life of most of them.

2. Phthisical patients as a group, are *relatively* insusceptible to influenza infection. This may be due to the tuberculous process itself or to some extrinsic but nearly related cause.

3. But many individuals with pulmonary tuberculosis *do* get influenza.

4. And the disease, having been contracted, in many cases hastens the fatal termination of the tuberculous process.

5. It may be that this phthisical exacerbation occurs more frequently in individuals with latent tuberculosis, individuals who are not at the time mobilizing their protective antibodies.

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—W. T. V.

### *What We Know and Do Not Know about Tuberculosis*

IT appears to be a law of our still rudimentary intelligence, that the less we know of a subject the more dogmatic we are inclined to be about it. This human trait, no doubt a part of the strategy of self-defense, is largely responsible for the perpetuation of ignorance and error. The mind which can free itself from prejudice and foregone conclusions, from assumption and assertion, is the mind which discovers new realities.

In nearly all departments of what we call medical science, our knowledge is so fractional that the term science has a flavor of satire; yet how con-

fidently we make our own sweeping unproved assertions, and how hotly we contradict each others'.

Opinion aside, what do we positively know about, for example, influenza, malignant disease, the complement deviation tests, vaccines, common colds, tuberculosis?

We have tried to analyze the status of this last subject, tuberculosis, from the point of view of the things we know—the things, at least, that we are agreed on at present—and the things we do not know, that is to say the things about which we disagree and debate. It is, of course, impossible to tabulate all we do not know: we have included only some of the more important points which call for solution.

*We know:*

That tuberculosis is infectious, parasitic in origin.

That it is caused by a specific organism, the tubercle bacillus.

That certain more or less characteristic tissue changes occur in its course.

That the conflict between bacillus and host often ends very early in favor of the host—so early that the presence of lesions is not suspected during life.

That in the majority of cases which are recognized clinically, the disease shortens life.

That its morbidity and mortality rates are high throughout the civilized world.

That there is a group of symptoms and signs, some or all of which occur in recognized cases.

That exhaustion and malnutrition in the host contribute to the development of the disease, while rest and adequate feeding favor recovery.

*We do not know, or do not know thoroughly:*

The biology of the parasite, how simple or complex, constant or variable, is its life cycle.

The significance of variations in the parasite, as the human and bovine strains, and their relation to each other and to the production of disease in various hosts.

The explanation of virulence and resistance, and the relative importance of these factors and of dosage in the development of disease.

The role of family and racial heredity, and of home exposure.

The relative importance of various modes of exposure and portals of infection.

The truth about such alleged predisposing factors as influenza and children's diseases.

The importance of carrier cases.

The physiology of defense, and the part played in it by body fluids and by special cells (e. g., endothelial leucocytes), tissues (e. g., lymphoid), and organs (e. g. adrenal).

The absolute and relative frequency of healed and active tubercle, and the amount of variation in these figures in different localities and classes, and under different conditions of housing, etc.

The occurrence of healed tubercle among savage races to whom tuberculosis is a new disease.

Whether infection, with invasion and some response on the part of the tissues, is practically universal in civilized races, whether it usually occurs in childhood, and whether an active immunity is commonly the result.

The effect of other diseases and of mental states on tuberculosis, and vice versa.

The effect of tuberculin, and the indications for its use in treatment, as well as the precise indications for rest, occupation, and diet.

The possibilities of protective vaccination and of chemical therapy.

The significance of mixed infections, and the factors concerned in toxæmia.

The effect of the antituberculosis campaign, and the relative importance of its various phases.

The difference in duration of life between the patients who follow our directions and those who do not.

As already stated, the list does not pretend to be exhaustive. It is rather illustrative of the little we know and the much we need to learn in order to act with confidence in the situations we have to meet. Our practical object is to reduce tuberculosis, our dream to eliminate it. Are our methods rational? Does our effort to keep the sick alive help or hinder the main achievement? Is it desirable to prevent exposure, or do we thus develop a vulnerable race, a race untrained in defense? These are questions which we cannot answer. All we can say is this: We may reason for the race and long for omniscience, but we must act from more immediate motives, and to the best of our present ability. We must use the little wisdom and skill we have to relieve and protect those who depend upon us here and now.

—G. B. W. and C. T. R.

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### *The Control of Measles*

BROWNLEE<sup>1</sup> recognizes the great difficulty in controlling or limiting the spread of measles and makes some suggestions which are of value. He states that among the middle classes in England it is observed that children rarely take this disease until the eldest in the family reaches the school age. This is not so markedly the case with the working classes. Among these, greater facilities of infection are afforded by the children playing together in the streets. In spite of this, however, in the opinion of a number of medical officers, the fire is lighted in the schools and the flame is carried to the streets. Under six months of age measles is not likely to be acquired, but when acquired the death rate is fairly high. Brownlee finds that most cases occur between the ages of six months and six or seven years. If it be assumed that in families of young children the children do not as a rule take measles until the eldest child has become sufficiently old to go to school, something can be done to prevent its spread, and this is suggested by Brownlee as follows:

<sup>1</sup>British Medical Journal, April 17, 1920.

"Each school will keep a register. When a child of five years is admitted to a class at school it will be noted in the record whether that child is the eldest, intermediate, or youngest member of the family. When measles breaks out in a class the register will be consulted. If the child exposed to infection be the youngest member of the family, nature may be left to take her way—there is no further danger. But if he or she be the eldest, especially if the younger children in the house are of ages between six months and three years, the direct action of nature is no longer a matter of indifference. What is a mild disease at the age of five years may be a matter of grave danger at the age of six months. Now it may be taken as practically certain that very few children develop the first symptoms of measles within seven days of the infection, and the higher limit may be set at fourteen days. If, therefore, the child who has been exposed to the infection stays in its home for seven days after exposure no harm will ensue. It is the next seven days which are important. In the encourage of families in towns, especially where the families are very young, the house of one grandmother is usually available. There are also quite frequently houses of uncles and aunts in which there are either no children or in which the children have already passed through the necessary attack of measles. It is thus only a matter of arrangement that the child who has been exposed to infection stays with a grandmother or other relative for a specified seven days. It may be objected that this cannot be done. On the contrary, I have made such arrangements for many years with reference to cases of scarlet fever and diphtheria. Where on dismissal there was some doubt as to whether a patient were free from infection or not, and where it was obvious that continued residence in the hospital was not the best way to clear the patient of infection, I at once appealed to the parents. I found that arrangements made were loyally carried out in nearly every case; the average parents are not selfish as regards their children, but much the reverse. There are, of course, a number of unreasonable and untrustworthy people, but in my dealings I have found these the exception. Once the matter has been carefully explained the ordinary person wishes to act for the best. Of course, at the institution of a new method of administration there will be a considerable amount of evasion, but from the moment that the opponents of the system see their children going to the grave while those who accede find their children do not take the disease, the sarcasm of the neighbors will affect more than thousands of regulations. A few years' trial will put all the community on your side, and then the work is done."

Brownlee emphasizes the fact that early diagnosis is a matter of prime importance in controlling measles. He states that there is no reason for believing that this disease is transmissible until the appearance of the first catarrhal symptoms. For early recognition purposes there are only two symptoms and signs that are of value. The usual advice is to look for Koplik spots, but by the time these are in evidence the disease has in all probability been transmitted. The temperature is one of the early evidences of this disease. When a child comes to school with suffused eyes and edema of the lower lids and is found to have a temperature, it may or may not be developing the measles, but it should be immediately isolated under the best conditions possible and kept in bed for at least four days. If it be measles, at the end of that time the eruption will appear.

Incidentally, Brownlee makes it evident that the case mortality in this disease is much greater among those treated in hospitals than among those treated in their homes. He gives figures for two cities, Aberdeen and Glasgow. In the former, most children with this disease are treated in their homes. In Glasgow, on the other hand, Brownlee's statistics include only those treated in hospitals. The case mortality in Glasgow is much higher at all ages than in Aberdeen. It may be that there is some other factor besides hospital treatment accounting for the difference in the death rates in the two cities, but this is the testimony nearly everywhere. Uncomplicated measles is a mild disease and has a low death rate, but the virus of this disease opens gateways to secondary infections, and these kill. Whether a child with measles will be more likely to escape secondary infection in its home or in a hospital depends upon conditions which must be judged by the medical man in charge. In a contagious disease hospital constructed and manned in a modern way the child with measles should find its safest place, but many such hospitals do not come up to this standard. The virus of measles is carried through the air for short distances, but in hospitals secondary infections are usually transported by careless attendants. The nurse in a measles hospital in going from one patient to another should exercise the same care and practice the same precaution as would be done if one of these children had measles and the other had scarlet fever. One child may harbor a deadly streptococcus and the careless attendant may carry this organism to every other child under her charge. Until the importance of preventing secondary infections in measles is understood and efficient methods of prevention are practiced the child with measles, under ordinary conditions, is much safer in an isolated room at home than it is in a hospital ward.

—I<sup>r</sup>. C. I<sup>r</sup>.



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## ORIGINAL ARTICLES

### PRACTICAL APPLICATIONS AND USES OF THE SCHICK TEST\*

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THE subject of diphtheria in many of its aspects is thoroughly familiar to the medical profession at the present time, but it may not be generally realized that in spite of the modern methods of combating this disease, it is still quite active and prevalent throughout the world. The mortality in New York City alone has been about 1,400 cases each year for the first past five years; the morbidity about ten times as great. For the United States the calculated yearly mortality is from 20,000 to 22,000 and the morbidity from 150,000 to 200,000 cases. In pre-antitoxin days the mortality from diphtheria was 70 to 75 per cent. With the introduction of antitoxin in 1894, the mortality was gradually reduced to 10 per cent, where it has remained more or less stationary.

The above are striking figures when we consider that we have at our disposal in diphtheria antitoxin a remedial agent which would cure every case if applied early enough in the course of the disease. The fact remains, however, that for the past eight to ten years the number of fatal cases has remained more or less constant. This persistent and relatively high mortality can only be accounted for by the delayed application for treatment on the part of the patient, the delayed recognition of the disease on the part of the physician, or by both of these factors.

The more recent investigations in the control of diphtheria have shown us the great importance of the Schick test by means of which we can recognize very definitely every person who is susceptible to diphtheria. In conjunction with the Schick test it has been found that the vast majority of susceptible individuals can be actively immunized for a number of years and possibly for life by means of injections of toxin-antitoxin. We realize fully that it will be a ques-

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tion of years before there will be a universal application of these measures, but it is a work well worth while and will repay the effort.

In 1913 Schick<sup>1</sup> published the results of his investigations in which he stated that by means of a simple clinical test we could determine whether a person is susceptible to diphtheria or not. The test consists in the injection of a small amount of diphtheria toxin, properly diluted, into the skin of the forearm. We began using the test at the Willard Parker Hospital soon after his publication appeared. After seven years of intensive studies with this reaction we have come to the conclusion that the test is one of the most valuable and accurate clinical procedures at our disposal today.

#### I. FACTORS INFLUENCING THE RELIABILITY OF THE SCHICK TEST

The reliability of the results obtained with the Schick test, however, depends upon three important factors, which have to be carefully observed:

1. The toxin that is used for the test should be of standard strength.
2. The technic in making the intradermal injections must be correct.
3. The interpretation of the reactions must be accurate.

The toxin used in the test must be of standard strength. The dilution must not be so weak that the individual who should give a positive reaction will give a negative reaction, and it should not be so strong that a local area of severe necrosis will develop at the site of the injection. Schick determined that the amount of toxin for the test should be 1/50 of a minimum lethal dose for the guinea pig, in 0.1 c.c. of normal saline. We prefer 1/50 of an M.L.D. in 0.2 c.c. of saline, an amount which is more easily handled, and will show a small definite wheal-like swelling in the skin after the injection. The positive reactions noted in susceptible individuals with this dilution of toxin are not apt to be as severe and persistent as those noted with the more concentrated dilutions.

The toxin can now be obtained in a convenient outfit devised by me and supplied by the Research Laboratory. It consists of a small glass capillary tube containing the undiluted toxin, a small rubber bulb for expelling the toxin and a 10 c.c. bottle of saline in which the toxin is to be diluted before use. The toxin remains good for six months if kept in the cold undiluted. After dilution it should not be used later than twenty-four hours. One outfit is sufficient for about 35 tests. Similar outfits are supplied by several commercial laboratories. I have found after testing some of these commercial preparations, that unfortunately a number of them were below standard strength. These results have been communicated to the laboratories, and I have no doubt that the strength of the toxin in these preparations will be brought up to the standard.

The outfit supplied by the New York State Department of Health is good, but is not fool-proof. In this outfit there is a small vial of toxin from which with a graduated glass pipette a definite amount is withdrawn and diluted with the saline supplied in another vial. One physician emptied the entire contents of the toxin vial into the saline, and tested some 200 children. About 80 of these children had severe sloughing reactions as a result of the concentrated dilution of the toxin which was injected.

When we make the tests with a standard and carefully prepared dilution of the toxin, our results show that the test is perfectly harmless. The informa-

tion obtained is of extreme value because of the great accuracy of the Schick reaction. Of 2200 scarlet fever patients who gave negative reactions on admission to the Willard Parker Hospital, not one developed an undoubted clinical diphtheria. These children had received no antitoxin, were exposed to cases of diphtheria in neighboring beds and moreover some 20 to 25 per cent showed by culture that they were carriers of virulent bacilli. The fact that all of the 2200 proved immune to the disease is a striking corroboration of the accuracy of the Schick test.

The technic of the test is of great importance. The reaction is a local phenomenon and has to be visible to the eye. The test fluid, therefore, must be injected *intradermally* and not subcutaneously. A good syringe and a fine needle are necessary. A 1.0 c.c. Record syringe and a 26 gauge  $\frac{1}{4}$  inch steel needle are probably best for this purpose. An ordinary hypodermic syringe will answer, but the needle should be finer than the ones that are generally used with such syringes. One must be careful to sterilize the syringe and needle by boiling, or with alcohol. If alcohol is used it must be rinsed out of the syringe with some of the diluted toxin and the rinsings discarded before making the tests. To make the test, inject 0.2 c.c. of the unheated and properly diluted toxin intradermally on the flexor surface of the right forearm about 2.5 inches below the bend of the elbow, and a similar amount of the heated diluted toxin used for control as described below in the left forearm. It is a good plan always to use the *right forearm* for the test and the *left forearm* for the control, as one will then be able to know exactly where to look for the reactions and how to interpret them.

The correct interpretation of the reactions is also of utmost importance. A *positive* reaction indicates that the child is susceptible to diphtheria; a *negative* reaction that he is immune to that disease. If a person is susceptible to diphtheria there will appear at the site of injection within 24 to 36 hours a definite well circumscribed area of redness about the size of a five cent piece, which gradually becomes more marked within the next two or three days. The reaction reaches its height on the fourth or fifth day. It persists for a week or longer, depending upon the intensity of the reaction, and leaves on fading a brownish area of pigmentation, which shows at first definite scaling. The pigmented area gradually fades, but may still be seen in some individuals even as late as three or four months. If a person is immune to diphtheria he shows a *negative* Schick reaction, the skin at the site of the test remaining unchanged. It has been found that if an individual has 1/30 of a unit of antitoxin or more per c.c. of serum, he will give a negative reaction. The reading of the reactions in children under 5 years of age is quite simple, as the reactions at this age are mostly positive or negative. In older children, however, and especially in adults, one meets with so-called *pseudonegative* reactions which make the reading more difficult. This reaction is found in immune individuals and must be carefully distinguished from the positive reaction. The pseudonegative reaction is produced by the autolyzed protein of the diphtheria bacillus, which is also present in the test fluid. This reaction is of the nature of an anaphylactic reaction. It appears much earlier than the positive reaction, is well marked at the end of eighteen hours and reaches its height at the end of twenty-four hours. At the

end of four days in a majority of the pseudonegative reactions most of the redness and the induration has faded away and there is left behind only a small irregular area of pigmentation, which may occasionally show a slight central scaling. These reactions vary in intensity, however, the degree depending upon the susceptibility of the individual to the autolyzed protein. Some of the pseudonegative reactions are very marked and may show even at the end of four days a well defined area of reddish brown pigmentation. The pseudonegative reaction, can, however, be accurately identified and distinguished from the positive Schick reaction by making a control test on the left forearm with toxin which has been heated to 75 °C. for ten minutes. The heating destroys the toxin, but does not affect the autolyzed protein. An individual who gives a *pseudonegative reaction* will show *similar reactions* in the *test* and in the *control*. An individual who gives a *positive reaction* will show an area of *typical redness* at the site of the *test*, which is always done on the right forearm, and *no reaction* at the site of the *control* on the left forearm. It is best to make a preliminary reading at the end of 48 hours and a final definite reading at the end of 96 hours. At the end of four days the positive reactions will be at their height, while the majority of pseudonegative reactions will have faded almost completely. The control test with *heated toxin* is most valuable, therefore, in positively identifying the character of the reactions.

A small proportion of susceptible individuals have a *pseudopositive* or *combined* reaction. This reaction represents a combination of both the positive and the pseudoreaction, and is identified at the end of 96 hours by a well defined positive reaction at the site of the test on the right forearm and a partly faded pseudoreaction at the site of the control on the left forearm.

The importance of identifying the pseudonegative reaction is evident from the fact that among adults there occur two or three times as many pseudonegative as positive reactions.

## II. ACTIVE IMMUNIZATION OF INFANTS AGAINST DIPHTHERIA

In the newborn we find only about 15 per cent of positive Schick reactions. This number corresponds with the number of mothers who give positive reactions. The other infants are temporarily immune. This immunity is derived from the mother and is generally lost after the first six to nine months of life. Most of the children by the time they complete the first year of life give a positive reaction. This explains the great morbidity and mortality from diphtheria between the ages of one and five years. Over 80 per cent of deaths from diphtheria occur under five years of age.

In view of this very high morbidity and mortality in children under five years of age we have urged that *every child* from six months to two years of age should receive the active immunization with toxin-antitoxin. This procedure is especially important as most of the children lose their maternal immunity when they reach the age of six to nine months. This susceptibility is readily shown by a positive Schick reaction, which is seen in such a large proportion of children at the age of one year. *In children under six months of age, toxin-antitoxin should not be used as it usually produces no immunization.* This is especially true in the first few months of life. After the child has passed its

second year of life the application of the Schick test is of great value in separating the children who are immune from those who are susceptible so that toxin-antitoxin injections may be given only to those who show a positive reaction.

### III. MIXTURES OF TOXIN-ANTITOXIN USED FOR IMMUNIZATION

In 1913, at about the same time that Schick published his investigations, von Behring proposed a new method of active immunization with diphtheria toxin-antitoxin. The use of such mixtures was not new, but their application in human beings was taken up for the first time by this observer and his co-workers.

Von Behring gave no exact details as to the methods by which he prepared these mixtures.<sup>5</sup> His communications were therefore of little value to us at the Research Laboratory in guiding us in the preparation of the toxin-antitoxin. Of the different mixtures with which we worked, we finally came to the conclusion that a slightly toxic mixture gave the best results.<sup>6</sup> At first we had to determine these results by taking specimens of blood and examining them for their antitoxin content. This was tedious and unsatisfactory and we then began to use the Schick test in conjunction with the toxin-antitoxin immunization.<sup>7</sup> The work was thus greatly simplified. We were able to select the susceptible individuals, give them the toxin-antitoxin and subsequently prove the development of a lasting active immunity by means of re-tests with the Schick reaction. The toxin-antitoxin injections consisted of 1.0 c.c. of the mixture given subcutaneously in the arm at the insertion of the deltoid and repeated twice at intervals of seven days. Not only was an active immunity produced in over 95 per cent of susceptible individuals, but we have found that this active immunity has persisted for five years, that is up to the present time and it is possible that it will continue for the life time of the individuals. This, of course, indicates that we have at our disposal today the means by which the susceptible part of the population can be protected, diphtheria controlled and finally eradicated.

The mixtures of toxin-antitoxin must be prepared in reliable laboratories and carefully tested. We have established a standard, which we believe it is safe to follow. The mixture should be of such strength that 5 c.c. injected into a guinea pig will produce a local induration at the site of injection followed by late paralysis, but will never cause acute death of the animal. Each c.c. of the toxin-antitoxin preparation supplied by the Research Laboratory contains 3 L<sub>1</sub> doses of toxin and 3.5 units of antitoxin. With a stronger toxin, which can occasionally be produced, mixtures of toxin-antitoxin can be prepared which will contain more than 3 L<sub>1</sub> doses per c.c.; for instance, mixtures of 4, 5, or 6 L<sub>1</sub> doses with a corresponding increase in the amount of antitoxin. Such mixtures are slightly more efficient. We considered it advisable, however, to adopt a standard strength. If desired, the dose of the mixture can be increased to 2.0 c.c., although even with the larger doses a few individuals will be found who seem to be refractory to active immunization. In such individuals we usually find that the giving of a second series of injections of toxin-antitoxin at the end of three months, when the Schick retest is made, will bring about an active immunity. Many thousands of injections have been given with the toxin-antitoxin preparation of the Research Laboratory *and not a single untoward*

*result was observed.* Through some unfortunate error one of the commercial laboratories sent to one of the southern cities a mixture of toxin-antitoxin which proved later to be highly toxic to the guinea pig. There were some eight or nine deaths among the injected children. Such an accident which is most deplorable is no argument, however, against the use of toxin-antitoxin. It emphasizes the fact that extreme care must be used in making and testing the mixtures of toxin-antitoxin, before they are sent out. According to our experience, however, mixtures that are safe when sent out will always remain safe with regard to their toxic strength and will be effective for immunization for at least six months.

#### IV. PRACTICAL APPLICATIONS OF THE SCHICK TEST

(a) The Schick test should be used in the home where a child has developed diphtheria. By testing the other children and adults the susceptible individuals are found. Each of these is then injected at once with a prophylactic dose of antitoxin in the usual way to produce an immediate passive immunity. The delay of forty-eight hours necessary for the reading of the Schick test has little danger connected with it and has the advantage of avoiding the injection of antitoxin into those who do not need it. However, in the case of young children who have been in close contact with the disease and who cannot be observed during the next forty-eight hours it will under certain conditions be safer to give prophylactic injections of antitoxin at once without waiting for the Schick test.

(b) During an outbreak of diphtheria in institutions and schools, the application of the Schick test will enable us to select the susceptible children, who may be only 20 to 25 per cent of the total, and immunize them with antitoxin. We shall thus be able rapidly to control an outbreak of diphtheria in an institution or school.

(c) The Schick test is of great value as a routine procedure in contagious disease hospitals, various other hospitals, and in many institutions and homes caring for children, such as orphan and infant asylums, day nurseries, public and private schools, etc.

(d) In private and institution practice it is important that the physician should know whether the children are susceptible or immune to diphtheria, even when there is no outbreak of the disease and there is no immediate danger. One very important fact should be emphasized. We have found that children over two years of age who give negative Schick reactions will continue to give such reactions over a period of years if the same technique and the same standard dilution of toxin is used in making the test. It seems, therefore, that such natural immunity persists for a long period of years and possibly for life. The fact that the negative Schick reaction in the naturally immune individuals indicates a long and persistent immunity adds great value to the use of the test in homes, institutions, schools, etc.

(e) In the routine prophylactic work in diphtheria, when there is no immediate danger from the disease, we should use the Schick test in conjunction with the active immunization with toxin-antitoxin. The work is so well established now that we had no hesitancy in asking the school authorities in New York City to allow us to apply the Schick test to all the children in 100 selected

schools. This work will be started on a large scale in the various schools in the different boroughs. With the consent of the parents the children giving positive reactions will be injected with toxin-antitoxin. Subsequently those receiving the immunizing injections of toxin-antitoxin will be re-tested with the Schick test to determine the development of an active immunity.

#### CONCLUSIONS

1. Diphtheria is a widely prevalent disease with a morbidity and mortality which have remained fairly constant during the past ten years.

2. Active immunization with toxin-antitoxin of all young children from six months to two years of age is essential in bringing up a diphtheria-immune population.

3. The Schick test and control test should be applied to all children over two years of age, and all those giving a positive reaction should be actively immunized with toxin-antitoxin.

4. The Schick test and toxin-antitoxin immunization will find great fields of usefulness in homes, in various schools, institutions, hospitals, etc.

5. Diphtheria outbreaks can be completely controlled in homes, institutions, and schools by promptly applying the Schick test and by giving prophylactic injections of antitoxin to the susceptible individuals.

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# OBSERVATIONS ON THE MOTILITY OF THE ANTRUM AND THE RELATION OF RHYTHMIC ACTIVITY OF THE PYLORIC SPHINCTER TO THAT OF THE ANTRUM\*

By HOMER WHEELON, M.D., AND J. EARL THOMAS, M.D., ST. LOUIS, MO.

IN a previous communication it was shown that the pyloric sphincter of the dog possessed the property of rhythmic contractility.<sup>1</sup> Unfinished observations at that time indicated that such motor activity of the sphincter was influenced by and coordinated with the activities of the antrum. The experiments here reported substantiate our previous statements relative to the sphincter and definitely show that the activities of the sphincter are influenced by those of the antrum.

## REVIEW OF BIBLIOGRAPHY

According to a number of observers the peristaltic waves of the pars media are differentiated from the characteristic motility of the preantral and antral regions. The more commonly accepted view, however, is that the entire course of a contraction wave of the stomach is simply peristaltic in nature and that it does not differ in character from similar waves in the intestine.

In 1886 Hofmeister and Schütz<sup>2</sup> studied the excised and bloodless stomach of the dog in a moist chamber. In such preparations a contraction wave appears a few centimeters from the cardia, moves peristaltically to a point about 2 cm. from the antrum at which point a deep, preantral contraction occurs, after which the "sphincter atri" contracts. This is followed by relaxation of the preantral contraction and a maximal contraction of the "antral sphincter" of sufficient strength to separate the antrum from the rest of the cavity of the stomach. During the height of contraction of the "antral sphincter" the musculature of the antrum as a whole contracts, the latter usually being preceded by a strong shortening of the antrum. Prior to this observation, Morat, 1882,<sup>3</sup> obtained graphic records of gastric motility by means of a large rubber balloon inflated with air. By use of the manometric method von Pfünge, 1887,<sup>4</sup> observed on an average three contractions of the antrum per minute, each of which lasted from 6 to 12 seconds. Von Moritz, 1895,<sup>5</sup> by means of an elastic balloon of moderate size attached to a sound passed through the esophagus, recorded the variations of gastric pressure and motility. According to this author the fundus and pyloric antrum show different functions: the former a digestive, the latter a motor function as shown by the appearance of rhythmical contractions at the rate of from 2 to 6 per minute. The works of Cannon, 1898,<sup>6</sup> and Roux and Balthazard, 1898,<sup>7</sup> clearly show that the pyloric portion is mechanically the most active part of the stomach. Radiographically the pyloric portion exhibits "peristaltic movements" throughout the entire

\*Read before the Physiology Club of the St. Louis University School of Medicine, St. Louis, Mo.



period of digestion. Luciani<sup>8</sup> states that "the movements of the pyloric antrum are set up in the part nearest the fundus (preantral contractions) and are mainly affected by the circular fibers." Duceeschi, 1897,<sup>9</sup> working on dogs previously provided with a gastric fistula and six hours after a meal found that a small balloon inserted into the pyloric antrum recorded a distinct form of rhythmic movements. These movements were characterized by contractions and relaxations in regular succession; each cycle being completed within 10 to 30 seconds.

J. Auer's<sup>10</sup> excellent paper on the motility of the rabbit's stomach strongly supports the contentions of the older school. He summarizes his results as follows: "From observations made on normal rabbits by inspection and by the amplification of these data by operative exposure of the stomach after the innervation of the viscus had been modified, it was found that a gastric wave of contraction is usually composed of two well-defined phases which, at the height of digestion, succeed each other in orderly fashion. During the first phase a contraction appears on the stomach near the esophageal insertion and travels peristaltically to the sphincter antri, apparently skipping in its course an angular section of the preantrum, the constriction at the beginning of the preantrum being maintained. During the second phase the sphincter antri contracts strongly, and during this contraction the rest of the antrum contracts *in toto*, moving towards the preantrum, and expelling the antral contents largely or entirely into the preantrum, causing the latter to bulge markedly. The antrum then relaxes slowly and with this relaxation the preantrum sinks away usually without signs of peristalsis." Hence, "a complete gastric wave of contraction in the rabbit seems to be produced by the orderly interaction of two more or less independent parts, one of which originates in the middle of the stomach near the esophagus, and the other in the neighborhood of the preantral muscle sling. Either part may occur without the other."

The latter statement of Auer would lead to the assumption of a dual set of excitors to motility in the two portions of the stomach, however, the work of Alvarez<sup>11</sup> has shown that under normal conditions the stomach follows gradients of irritability, rhythmicity and latent period from the cardia to pylorus.

Cole,<sup>11</sup> from a study of serial radiographs of the human stomach, has shown that the sphincter bears a definite relationship to the antrum. That is, the amount of contraction of the sphincter is in proportion to the activities of gastric waves. When the gastric peristalsis is feeble the contractions of the sphincter are weak; when the gastric movements are strong, the sphincter is more strongly contracted. He has further shown that during the "systole" or active phase of every gastric cycle the pyloric ring is open and a small amount of gastric contents is propelled through its lumen into the "reservoir cap." The "terminal peristaltic wave" which has meanwhile been advancing toward the pyloric ring, upon reaching the sphincter, effects its closure so that the lumen is entirely obliterated, or visible only as a thin line.

The paper by Luckhardt, Phillips and Carlson<sup>12</sup> is of special interest at this point. They observed both graphically and fluoroscopically that in man the "pylorus" opens for the ejection of chyme with the arrival at the pyloric sphincter of powerful advancing rings of contraction aided possibly by a general

increase in tone of the musculature of the stomach as a whole. In dogs the gastric content was observed to issue from a duodenostomy either during a marked rise in gastric activity, or more commonly just at or after the peristaltic wave passing over the stomach had affected its greatest increase in intragastric pressure. They further demonstrated that a more definite relation exists between the muscular activity of the stomach and the opening of the pyloric sphincter than between the opening of the sphincter and the reaction of the gastric contents. In discussing their observations in relation to the findings of Alvarez, they state: "With a gradient higher in the body of the stomach than near the pylorus, the increased pressure coming from above effects the opening of the pylorus even before the free acidity has reached a concentration sufficient to assure chemical control of the sphincter." Their graphs are most instructive and convincing. Just prior to the above report Ivy<sup>11</sup> made the observation that water issued from the dog's stomach in such a manner as to indicate a dependence upon the peristaltic waves in the stomach.\*

Such observations definitely establish the antrum as an organ of special motility, the purpose of which is to mix the ingesta and bring it into contact with the gastric juices, and to propel the semidigested chyme onward into the duodenum. Save for the observations of Cole and Luckhardt, Phillips and Carlson, but little definite work has been done on the relation of motility in the antrum to that of the sphincter. The lack of information concerning the motor relation between the antrum and sphincter is, no doubt, the result of the usually accepted theory of an "acid control of the pylorus." Cole drew his conclusions from a study of serial radiographs of the human stomach. Luckhardt, Phillips and Carlson observed the egress of gastric contents from a duodenal fistula in relation to pressure changes in the stomach. Their results were also checked radiographically, both in the dog and man. Many observers have used the balloon method to study motility in the filled or empty stomach. However, we have failed to find any record of simultaneous tracings obtained from the antrum and sphincter. The motor relations between the sphincter and antrum, here reported, are the results of studies of simultaneous tracings obtained from these two parts.

#### FUNCTIONAL ANATOMY OF THE PARS PYLORICA

A brief description of the structure of the terminal portion of the stomach seems to be justifiable in order to make clear the results obtained by graphic procedures.

The pars pylorica of the dog's stomach is structurally the equivalent of a vent the diameter of which diminishes progressively toward the pyloric sphincter. (Fig. 1.) The inner wall of the right portion of the stomach shows the orifice which constitutes the entrance to the pars pylorica clearly defined. The upper rim of the orifice which is coincident with the incisura angularis forms a definite ring which in its course becomes attenuated to aid in the formation of the wall of the vestibule on the greater curvature. The angle of descent of the

\* Since the publication of this paper McClure, Reynolds and Schwartz,<sup>12</sup> from fluoroscopic studies, assert that under normal conditions the human pyloric sphincter opens regularly at the approach of each antroduodenal peristaltic wave as chyme to pass through into the duodenum during an appreciable length of time, and that the antroduodenal peristaltic wave has spent itself. They further report that acid is not the controlling factor in the opening and closing of the pyloric sphincter in man.

fibers in this structure decreases to a point opposite the incisura angularis. By reason of this distribution of the circular fibers the vestibule or preantral region assumes the form of a triangle the apex of which is at the rim of muscle forming the incisura. This condition is graphically shown in Fig. 1, in which the triangle is indicated as formed by that portion of the stomach lying within points I A, X and C. The pars pylorica, or outlet of the stomach, therefore, may be considered as arising at the incisura angularis on the lesser curvature and a point opposite on the greater curvature where the circular fibers lie in a plane transverse to the long axis of the antrum. The terminal portion of this region is represented by an accumulation of circular fibers which form the pyloric sphincter, or valvula pylori, B.N.A. In the majority of the dogs observed the

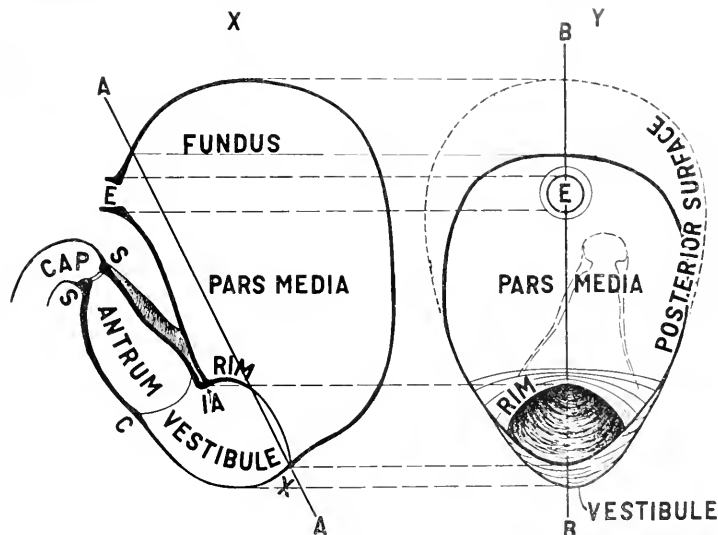


Fig. 1.—Diagrams of the dog's stomach. X Outline of posterior half of stomach. A Line of incision of the fundus to the vestibule, antrum and sphincter. S Cap. Duodenal con. opening into the small intestine. A.A. Line of incision of stomach to show the right half of the stomach. Note the position of the outlet and the relation of the vestibule to the antrum.

musculature of the sphincter was accentuated on the greater curvature. The pyloric canal is a rather variable factor, although it is usually described as the aperture leading from the terminal portion of the antrum into the duodenum. Functionally the extent of the canal is determined by the amount of muscle involved in an antral contraction plus sphincteric action for, as will be shown later, the pyloric ring only represents an accumulation of functional units of the terminal portion of the antrum.

#### EXPERIMENTAL METHODS

The observations here reported were made upon 44 operated and normal dogs. All operative procedures were carried out under ether anesthesia.

Graphic records were obtained both on the anesthetized and conscious animals following the operation at various intervals. Briefly, the operation consisted of passing an enterograph into the pyloric canal and antrum through an incision in the fundic portion of the stomach. After securing the recording apparatus in position the opening in the stomach was closed by a purse-string suture about one or more tubes leading from the placed apparatus. Aseptic precautions were observed in all cases in which the animal was permitted to live for a period longer than 12 hours.

The various types of apparatus used for obtaining graphic results of antral

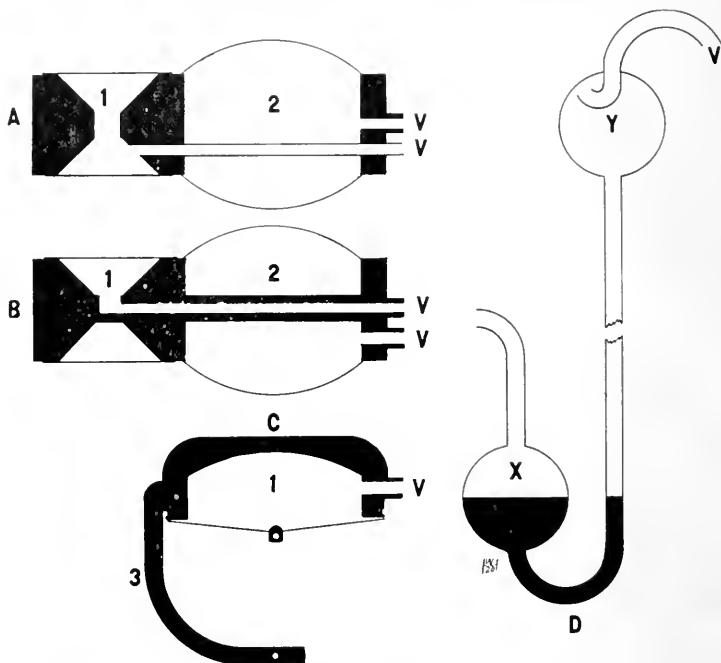


Fig. 2. Diagrams of apparatus. *A*, Double flexible enterograph. *B*, Rigid or spindle enterograph. Air chamber in pyloric canal; 2, in antrum. *V*, Vents leading to water manometers; *C*, tambour myograph showing air chamber (1) and vent (1); 3, rigid arm for anchorage of sphincter or segment of gut. Condon rubber used for chambers 2 of *A* and *B*, finger cot for chambers 1 of *A* and *B*. *D*, Water manometer. *X*, reservoir bowl containing water; *Y*, check bowl for receiving water should pressure force column to such a height; *V*, vent leads from chamber *Y* in such a manner as to prevent easy exit of water from bowl. This manometer was found to be of special use in obtaining traces from the human stomach.

and sphincter action are modifications of the pylorograph described in our previous paper.<sup>1</sup> For the sake of convenience we have termed these modified balloons enterographs. In brief they consist of two chambers, one of which occupies the pyloric canal; the other the antrum. The details of construction are shown in Fig. 2. The balloon chambers are connected by means of heavy rubber tubing with water manometers (Fig. 2-D) carrying from 5 to 20 cm.

of water pressure for balloon distention. Piston, bellows, and tambour recorders were used for registration of motility. Save for the water manometers air transmission was used entirely.

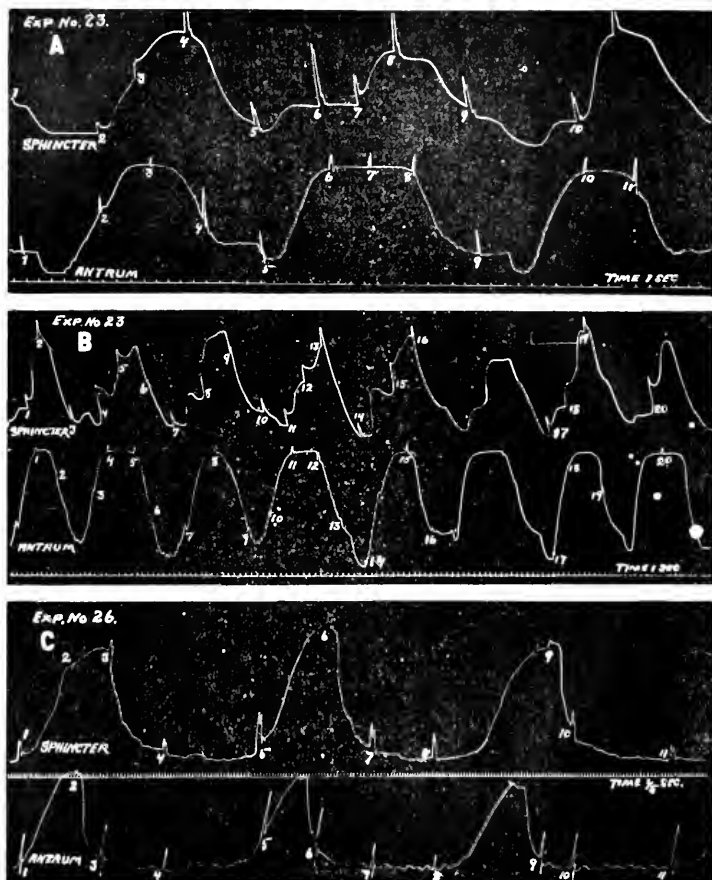
#### EXPERIMENTAL RESULTS

(A.) *Direct Observations on the Exposed Stomach.* In the filled stomach exposed in a warm saline bath, waves of peristaltic contraction arise in the region of the pars media on the greater curvature. The constriction band then travels toward the antral region gaining in strength as it progresses. The depth of contraction is often greatest just in front of the vestibule at which point the contraction is often completed. However, the phenomena, which usually follows the arrival of a constriction band at the vestibule is a slight wave of contraction, barely visible to the naked eye, which sweeps over the entire vestibule or "preantral sling" to begin again as a powerful, progressive peristaltic wave which passes along the first part of the antrum. At times a peristaltic wave passes progressively from its origin over the vestibule and first portion of the antrum. We have seen a condition analogous to that described by Auer<sup>29</sup> in the rabbit in which a peristaltic wave reaches the preantral region either to cease or, after a pause, to manifest itself again in the first portion of the antrum. We have also noted antral action independent of fundic action. However, we are of the opinion that the continuous wave represents more nearly the normal movement of the moderately filled dog's stomach.

The appearance of a peristaltic wave in the region of the antrum is followed by antral activity. This motility of the antrum which begins at the right of the incisura angularis continues as a progressive movement which finally terminates in the closure of the sphincter. Therefore, a wave of motility in the dog's stomach may progress from its origin over the entire gastric surface to terminate in the closure of the sphincter. The longer diameter of the antrum was observed to contract just preceding the beginning of contraction of the circular fibers, but a simultaneous contraction of the antrum *in toto* was not observed by us. However, we have seen the entire antrum in a state of contraction sufficient to cause marked anemia of the parts. This anemia begins in the gastric end of the antrum and gradually increases in extent until the entire antrum is bloodless. At such a time the sphincter begins to lose color and this loss of color persists during the return of blood to the gastric portion of the antrum or at a time of antral relaxation. This distribution of blood in the pars pylorica is analogous to the tracings to be described later, i.e., antral contraction followed by sphincter contraction. Inhibition was observed in the antrum as a whole just before the beginning of its active phase, but was never observed to occur in antral fibers caudal to the point of constriction. On the other hand, marked relaxation follows behind the contracting portions. So marked is this wave that it might well be called an advancing wave of relaxation, which progressing over the antrum and sphincter return these structures to their quiescent phase.

(B.) *Graphic Observations.*—1. Rhythmic Motility of the Antrum.—The antrum, like the pyloric sphincter, demonstrates rhythmic activity. The in-

distinct cycles consist of phases of contraction, relaxation, quiescence and inhibition. These phases follow each other in an orderly sequence and are maintained for hours both in the filled and recently emptied stomach. These cycles appear at the rate of from 3 to 5 per minute, that is, each cycle re-



quired 12 to 20 seconds for the 100% contraction. The rhythmic contractions of the 100% are not lost because of 100% ether anesthesia, or following decapitation of the entire stomach.

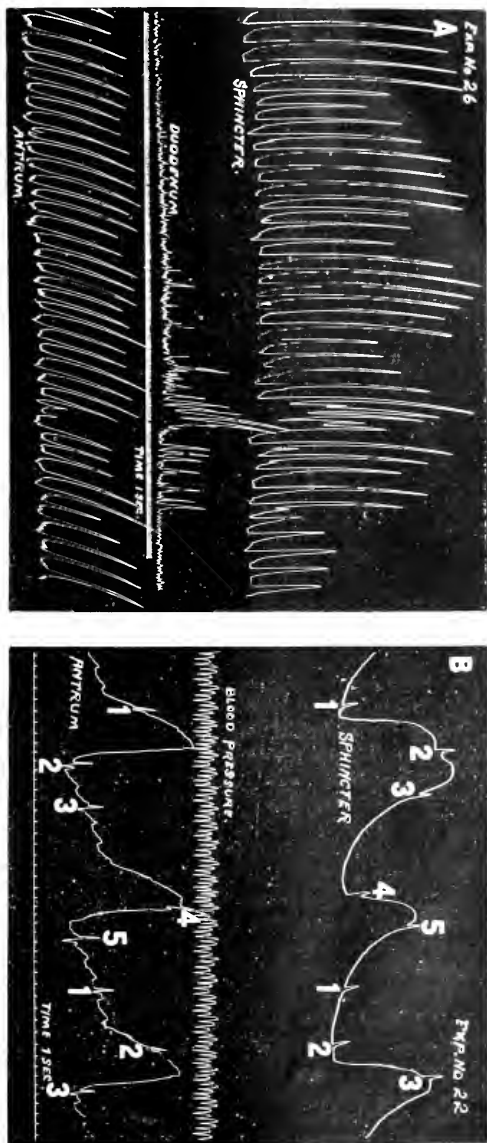


Fig. 4. Tracings showing relation of antral to sphincter motility. *A*, Experiment 792, February 14, 1950. Conscious animal. Traces obtained with a high electrograph. Note alterations in antral and sphincter curves during diarrheal activity. *B*, Experiment 222, February 1, 1950. Ether used during taking of records. Large antral bulbous. Note relation of antral to sphincter motility.

Characteristic antral cycles are shown graphically in Figures 3 and 4. The antral cycles shown in these two figures while analogous in character, demonstrate certain variations. The trace in Fig. 4, *B*, was obtained by using a flexible enterograph with a rather large balloon in the antrum. Such a balloon of necessity occupies the entire antrum and possibly a portion of the preantral region, hence it will transmit pressure changes occurring in the region occupied from the very beginning and throughout the contractile period of the entire antrum. With such an apparatus the greatest degree of depression in these cycles occurs as the final phase of relaxation. From this point on, tone is gained, superimposed upon which appears the phase of contraction. The wave of contraction is rapidly completed and does not demonstrate a holding phase, possibly, because of reduced pressure (inhibition or relaxation) in the preantrum and beginning portion of the antrum. Such graphs are very similar to those obtained with a free balloon in the partially filled or empty stomach. The antral tracings of Fig. 3 are typical of those obtained with a spindle enterograph, a small balloon, firmly held in position, constituting the antral portion. Such graphs demonstrate a plateau or sustained contraction following the initial rise of the lever. The plateau gradually declines at the end of the contraction phase and then the lever falls rapidly to reach its lowest point or a quiescent phase which is soon followed by a definite wave of inhibition. This latter type of response, which is well shown in Fig. 3, *A*, was the one most commonly observed in our series of experiments. In those cases where the greatest degree of relaxation follows immediately upon the contraction phase there appears a gradual upward climb of the lever, however, this is usually broken by a wave of inhibition which occurs just prior to the appearance of the positive phase.

II. Motility of the Pars Pylorica.—Direct observations of the pars pylorica at a time of active motility are difficult to interpret. However, a double enterograph, one chamber of which is inserted into the pyloric canal, the other into the antrum, gives definite information as to the functional relation of the antrum to the sphincter. Recording levers attached to the chambers of the enterograph demonstrate rhythmic activity. The movements in the two portions, however, do not occur synchronously, but they do occur rhythmically and at definite intervals of time, depending upon the distance between the two receiving chambers of the enterograph. The two levers do not necessarily bear any definite relation to each other in their degree of oscillation, although they are placed so as to give the same degree of magnification and are working under the same pressure. However, the movements of the antral lever are always more regular and less variable than those of the sphincter. At no time did a contraction of the antrum occur which was not followed by some motor responses of the sphincter (Fig. 4, *A*). Such a response might have been anticipated in view of the fact that the circular fibers constituting the sphincter represent a piling up at a definite point of the circular coat of the antrum itself.

Incidentally it may be stated that we frequently had occasion to observe the influence of abdominal and gastric incisions upon the gastric motility. It was found that while such manipulations modified gastric motility as to degree



they did not abolish it and, in fact, appeared not to interfere seriously with the emptying of the stomach. It will be noted that these findings do not agree with observations on other laboratory animals, e. g., those of Auer<sup>17</sup> on the rabbit. So far as we could determine by the methods employed the degree of motility of the antrum and sphincter was not perceptibly affected by ether anesthesia of a moderate depth. With the development of a deep, surgical anesthesia there resulted a gradual reduction of motility. Complete loss of motility and residual tonus could be obtained only under excessive doses of the anesthetic.<sup>1</sup> Graphic results obtained while the animal was under a light anesthetic and at various times following recovery from the anesthetic were of the same type and nature. However, our best results were obtained from dogs sufficiently etherized to barely abolish voluntary reflexes.

The time relations of the activities in the antrum and sphincter are shown graphically in the tracings of Figures 3 and 4. In these tracings it will be noted that the antrum, like the sphincter,<sup>1</sup> demonstrates cycles of rhythmic motility. The antral cycles show the same duration as those of the sphincter, however, the duration of the phases is not the same in the two structures.

The tracing of Fig. 4, *B* was obtained with a double enterograph, a large balloon constituting the antral chamber. In this tracing the antral contraction has maximally relaxed at the moment of complete contraction of the sphincter, numerals 2, 5 and 3. The sphincter goes into action only after the antral contraction is well started, numerals, 1, 4 and 2. The maximal relaxation of the sphincter in this trace occurs during the active phase of the antrum, on the contrary the antrum shows its greatest degree of relaxation at a time when the sphincter is actively contracting. Immediately following complete relaxation the antrum gains tone up to the point of a second positive phase. During this time the sphincter is rapidly relaxing. Hence, the trace demonstrates a constant type of alteration in the phases of the two parts. Such graphs have been taken for hours, both on the anesthetized and conscious dogs without showing alterations in the response between the antrum and sphincter. The three tracings shown in Fig. 3 were obtained with a rigid, double enterograph, a small balloon constituting the antral chamber (Fig. 2, *B*). Inhibition in the antrum preceding a contraction is well shown in the trace *A*, and in the last 4 cycles of trace *B*. In trace *C* no definite phase of inhibition is shown preceding the phase of contraction. A similar condition holds for the sphincter. In this trace a decided phase of quiescence is common to both antrum and sphincter, although the active phases in the two parts are the same as those described above. In traces *A* and *B* the sphincter demonstrates a more or less well defined relaxation or inhibition which occurs during the active phase of the antrum. In the graphs of Fig. 3 and Fig. 4, *B*, the sphincter demonstrates its active phase in such a manner as to reach its maximum while the antrum is relaxing, also the sphincter shows its greatest degree of relaxation during the first portion of the active phase of the antrum.

The irregularities in the sphincter curves as shown at points 6 to 7, and 10, of Fig. 3, *A*, and those of trace *B*, of the same plate appear to result because of activities of the duodenum. During the registration of trace *C* the duodenum was observed to be in a quiescent state, and it will be noted that the rise and

fall of the sphincter curve is uniform. Later in the experiment, however, the duodenum did demonstrate rhythmic or segmental action for a short period, and during this period alterations occurred in the activities of the sphincter, Fig. 4, A. In spite of the variations in sphincteric action the relation of its major activities to that of the antrum is maintained. The relation of duodenal activity to that of the antrum and sphincter is now under consideration and will be reported in a later paper.

Results of a similar nature were obtained by the use of a tambour myograph (Fig. 2, C) applied to the exterior of the sphincter and a balloon of moderate size in the antrum (Fig. 5). In this case, as elsewhere, the antral contraction is followed by a sphincteric contraction which reaches its height during the period of relaxation of the antrum.

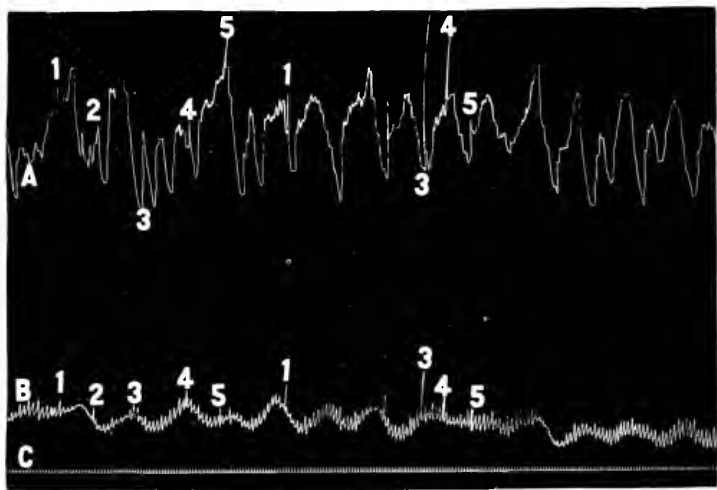


Fig. 2. Experiment 27; February 19, 1920. Ether used throughout. Animal immersed in tank of warm saline. A. Sphincter contractions obtained with tambour myograph (Fig. 5). Because of the structure of the myograph the down stroke in the trace represents contraction. B. Gastric motility obtained by using a small tree balloon in the stomach.

The results of our graphic observations are similar in nature to those obtained by direct observation of the stomach, namely, blanching of the antrum during its positive phase followed by loss of color in the sphincter when it enters upon its contraction phase. This loss of color in the sphincter occurs at a time of rapid return of color in the antrum; the flush in the sphincter appearing again with the blanching of the first part of the antrum.

Such results definitely show that the antrum enters upon and reaches its height of contraction at a time when the sphincter is relaxing or quiescent and that the sphincter goes into action some time later to reach the height of its contraction at a time when the antrum is beginning to relax or is rapidly relaxing. A period of quiescence, or relaxation, is then common to both antrum

and sphincter. The sphincter and antrum both invariably show some degree of relaxation (inhibition) immediately prior to their active phases: the sphincter demonstrating inhibition during the active phase of the antrum, and the antrum during the period of relaxation of the sphincter. Hence, the activities of the antrum and sphincter taken together may be considered as constituting a *cycle of the pars pylorica*.

The duration of the phases, as indicated above, is not the same in the two portions of the pars pylorica, neither do they develop their maximal activity simultaneously. The sphincter contraction begins at a time when the antral contraction is already practically maximal and does not reach its maximum until the antral contraction has disappeared. That is, the phase of contraction of the sphincter covers the period of sustained contraction and the greater portion of the relaxation phase of the antrum. The sphincter, therefore, acts in such a manner as to cover a negative phase in the antrum and add, on the average, 2 and  $\frac{1}{2}$  seconds to the positive phase of the pars pylorica. Thus it will be seen that the sphincter's contraction serves to supplement that of the antrum and to guard against the return of material that has been forced into the duodenum by the preceding antral contraction. Inhibition of a more or less marked degree immediately precedes the development of the positive phase of the cycle in both the antrum and sphincter. In the sphincter the occurrence of this inhibition is usually simultaneous with the development of the active or contraction phase of the antrum. This inhibition of the sphincter may be regarded, therefore, as an example of Langley's law of the intestine since it removes the tonus of the normal antagonist of the antrum and facilitates thereby the antral function.

If the above conclusions are justifiable then the activities of the two parts (antrum and sphincter) must be coordinated and to a high degree dependent upon each other. In other words, the activity of the lower segment should depend upon the reception of waves of excitation and inhibition passed into it from the segment above. The results reported appear to justify such conclusions, however, the following experiments were performed to determine whether or not such a condition subtends for this region of the stomach.

Immediately following the operation for the placement of a pylorograph (surgical anesthesia) and while the stomach was in a state of inactivity the effects of electrical stimulation of the antrum upon the sphincter were noted. Graphic results of such procedures are shown in Fig. 6. Curve 3 in this figure represents the motor response of the sphincter to an electrical stimulus applied to the stomach 2 and  $\frac{1}{2}$  inches from the sphincter. This curve, obtained by the use of the pylorograph, demonstrates a characteristic wave of contraction and relaxation after a latent period of 4 and  $\frac{1}{2}$  seconds. A similar curve is shown at 4 in the same figure. The small secondary responses in these two tracings were observed to occur in conjunction with a wave of motility in the first part of the duodenum. Direct stimulation over the sphincter also caused it to contract, usually after a latent period of but 1 to 2 seconds, curve 5. Similar results were obtained following stimulation of the first part of the duodenum, curves 6 and 7. Results of the same nature may be obtained on the active stomach.

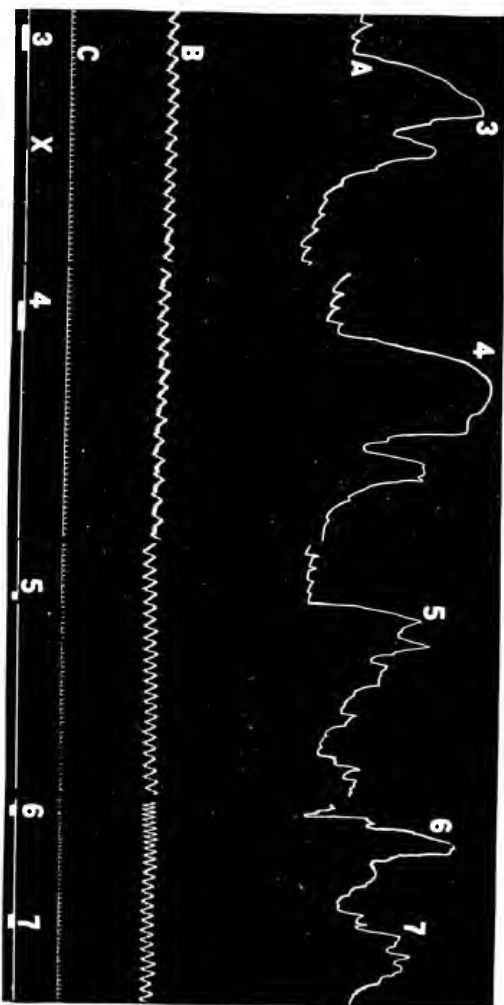


Fig. 5. The top showing the results of gastric stimulation. *A*, *A*, starts of the quiet stomach (1000 cycles per minute). *B*, *B*, stimulation of the stomach 21½ inches from the pylorus. *C*, *C*, stimulation of the stomach 21½ inches from the pylorus. *A*, *A*, stimulation above sphincter, *B*, *B*, stimulation above sphincter. *C*, *C*, stimulation above sphincter. All other points are from a nonrhythmic stomach. *B*, *B*, Blood

This experiment, which is typical of the series of similar observations, seems to point to a motor control of the sphincter because of impulses transmitted to it through the antrum. However, this experiment does not directly show inhibition of the sphincter as the result of antral activity. This may be accounted for upon the assumption that a quiet and more or less atonic preparation fails to register other than contractile phenomena. Therefore, inasmuch as a definite latent period exists between the application of a stimulus to the antrum and a positive phase in the sphincter, the length of the latent period depending upon the distance of the electrode from the sphincter, it may be assumed that the sphincter is not excited because of the stimulation applied to the antrum but by the transmission of a positive wave of contraction which passes over the pars pylorica.

III. Radiographic and Graphic Results.—The above reported results, if correct, should be observable in the unoperated animal by means of the x-rays. That is, if the antrum and sphincter demonstrate coordinated motility, radiograms taken at short intervals during the passage of an opaque meal from the stomach should show a constant relation in the distribution of the meal during the same phase of activity in any given part of the stomach. That this is so for man has been clearly shown by the studies of Cole, Luckhardt, Phillips and Carlson who observed fluoroscopically the exit of an opaque meal in dogs provided with a duodenal fistula, also found that the activities of the sphincter bore a definite relation to those of the antrum.

The three drawings in Fig. 7, A represent the form relation of the antrum to the sphincter during a characteristic cycle of the pars pylorica. In No. 3 the antrum is shown pouring its contents through the widely relaxed sphincter into the duodenum, the size of the antrum is much reduced, and the sphincteric canal and duodenum are engorged with the barium mixture. Following this stage the sphincter actively closes as is shown in No. 4, by the narrow hairline of barium connecting the duodenum and antrum. At this time the antrum shows rapid relaxation as indicated by the increase in size and loss of tonicity. Relaxation of the antrum continues from this point on as is shown by the changes in the position of barium in this region and the stomach as a whole. In the last cut the stomach as a whole demonstrates an inactive phase although the duodenum shows active segmentation. The sphincter during this time of complete antral relaxation (diastole) is free of barium, as shown in No. 5. These three drawings from a series of successive radiograms obtained from a normal dog's stomach are equivalent in nature to the results obtained by direct observation and by the use of graphic means; that is, the antrum is actively contracting to pass material into the duodenum at a time of quiescence on the part of the sphincter, and the sphincter is closed during the period of beginning relaxation of the antrum; an inactive period associated with different tone levels then being common to both.

The relation of the sphincter to the antrum was further studied by combining radiographic and graphic methods. This was accomplished graphically by securing an open pylorograph—the equivalent of an open cylindrical balloon—in the pyloric canal.<sup>1</sup> This device permits the recording of the sphincter's motility and at the same time allows the stomach to pass its contents into the

small intestine. The animal was permitted to recover from the operation, (usually twenty-four hours), at which time graphic records were begun. Following this a barium mixture was given by means of the stomach tube. With the graphic method it was possible to take radiograms at will, of any phase of the sphincter's action. Such radiograms not only show the position of the sphincter but also the position of the barium in all parts of the stomach and

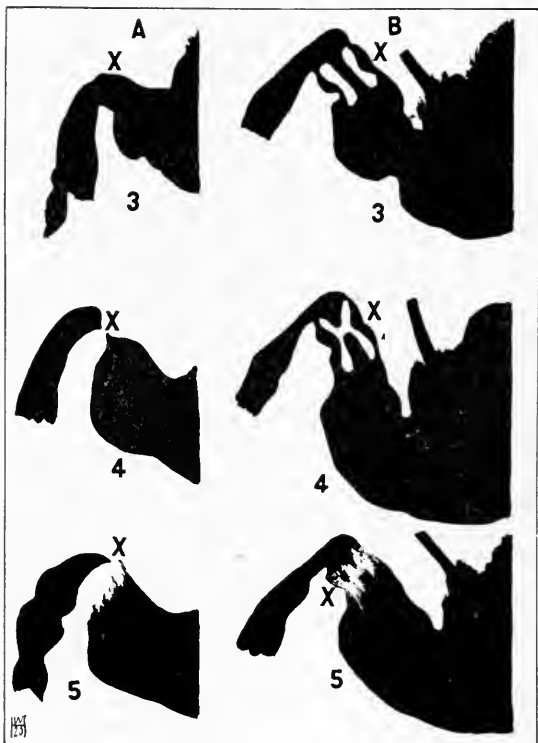


Fig. 7.—Drawings from radiograms showing the relation of the antrum to the sphincter. *A*, Experiment 32; February 18, 1920. Normal dog starved 5 hours. *B*, Experiment 24. Barium mixture by tube 24 hours after placing open pylorograph in the pyloric canal. Animal conscious. Light areas indicate air chamber of enterograph. In *B*, 3, barium is passing through tunnel of enterograph. In 4 the inner walls of the tunnel are shown cutting off the passage of barium from the stomach. In 5 the apparatus outline is indefinite; barium in antrum under low tension. *X*, Sphincter.

duodenum. Hence, with this method it was possible to obtain graphic tracings which were characteristic for the sphincter, and also radiograms which were identical in principle to those shown in Fig. 7 from the normal stomach.

The tracings in Fig. 8 are graphic records of the sphincter's action as obtained with the open pylorograph. The numerals 3, 4 and 5 indicate points

at which radiograms were taken. The radiograms are reproduced in Fig. 7, *B*, Nos. 3, 4 and 5. Radiogram 3 was taken just before the lever of the recording apparatus began to ascend, or at a time of active contraction of the antrum. Picture 4 was taken just as the pyloric sphincter reached its highest point of contraction, Fig. 8, No. 4, and at a time of beginning relaxation of the antrum (Fig. 7, No. 4). It will be noted that the ring of contraction in No. 3 of Fig. 7, *B* is not present in No. 4, also that the line of barium passing through the tunnel of the pylorograph is discontinued in No. 4. This condition, therefore, is the equivalent of that shown in Fig. 7, *A*, No. 4 which was obtained from a normal stomach. Picture 5 of Fig. 7, *B*, was obtained immediately following relaxation of the sphincter as shown by the recording lever, Fig. 8, No. 5. This

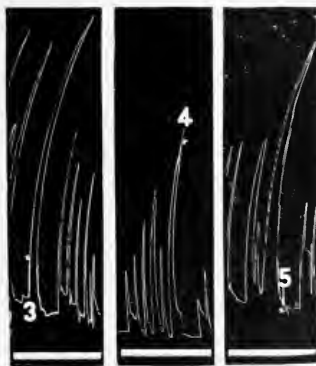


Fig. 8.—Experiment 24; February 9, 1920. Tracings obtained by use of open pylorograph. Radiograms in Fig. 7, *B* 3 4 5, were taken at points marked 3, 4, 5. Time  $2\frac{1}{2}$  seconds.

cut which is the equivalent of No. 5 in Fig. 7, *B* shows the antrum and stomach as a whole to be in a state of relaxation or quiescence preparatory to a second antral cycle. Barium is not present in the tunnel of the pylorograph, hence, antral pressure is not sufficiently strong to force material forward during its quiescent phase. The same condition due to sphincter tone is shown in No. 5 of Fig. 7, *A*.

These results seem to us of special importance for they clearly show that the activities of the stomach are not materially altered because of the presence of a foreign body in the pyloric canal through which the gastric contents may pass. Also the results of this procedure are equivalent to those obtained by other procedures. Therefore, the relationship between the activities of the sphincter and antrum above described may be taken as indicative of the normal relation existing between these two parts of the stomach.

#### DISCUSSION

Sources in error in the interpretation of results due to operative procedures have been eliminated only to the extent to which the results obtained by means of the x-rays may be relied upon. According to our results there is apparently no

qualitative difference in the movements of normal and operated stomachs if sufficient time is allowed for the latter to resume activity following operation. Our observations are in accord with the prevalent idea that the intestines may remain motionless for some time following laparotomy. On the other hand, movements begin in the stomach of the dog shortly after the abdomen is closed. The presence of a foreign body in the antrum and pyloric canal may influence motility. The results here reported seem to indicate that the antrum reacts in the same manner in the presence of a balloon as it does in the presence of food. We feel that the presence of the apparatus does not alter the nature of gastric motility.

The results obtained from observations on the excised stomach or from graphic records obtained by means of balloons or surgical procedures, it is true, are difficult to interpret and are open to severe criticism. The inability of a free elastic balloon in the stomach to properly transmit gastric motor changes has recently been emphasized by Luckhardt, Phillips and Carlson.<sup>12</sup> In conformity with their statements, we were unable to show by such a method any degree of uniformity between graphic records and radiographic findings. This is especially true when the stomach is partially filled, because at such a time the balloon will rise to the surface of the gastric content. This was observed on experimental animals and also in human subjects. However, a small balloon or enterograph secured in the antrum and carrying a pressure equal to from 5 to 15 cm. of water will transmit rhythmic oscillations to a recording apparatus, the results of which are analogous to those observed directly and radiographically. Such oscillations, as described by Duceschi, 1897, appear rhythmically and consist of cycles composed of a contraction, a relaxation, a quiescent and an inhibition phase. Such contractions, like those of the sphincter, are maintained for hours in both the conscious and anesthetized animal whether the stomach contains food or has been recently emptied.

Since the work of Cannon<sup>13</sup> the opening and closing of the "pylorus" has been ascribed to an acid control. However, Duceschi<sup>13</sup> had previously shown that the introduction of a 0.15 per cent hydrochloric acid solution into the vicinity of the cardia and fundus excites typical peristaltic waves. In the region of the antrum a 0.10 per cent solution produced a delay in the rhythm while stronger solutions weakened the intensity of antral contractions and altered their course. The theory of an acid control of the pylorus has never explained certain phenomena associated with the emptying of the stomach. The same is true of the theory of fluidity. Certain clinical and experimental observations have definitely shown that factors other than acid are directly concerned in the phenomena of gastric evacuation. Neilson and Lipsitz,<sup>14</sup> for instance, have shown that posture to a great extent determines the time of retention of water in the stomach. Furthermore, Spencer, Meyer, Rehfuess and Hawk<sup>15</sup> have shown that a 1 per cent solution of sodium bicarbonate hastens the emptying of the stomach either by increasing its motility or by opening of the "pylorus." They have further shown that hydrochloric acid is not necessary for the opening of the stomach for it empties, at times, when its contents are alkaline.



The radiographic studies of Cole,<sup>11</sup> while apparently not undertaken to disprove the theory of an "acid control of the pylorus," has done much to arouse suspicion concerning it. According to this observer the amount of contraction of the pylorus is in proportion to the activity of the gastric peristalsis, that is, when the gastric peristalsis is feeble the contraction of the sphincter is weak, and when the gastric peristalsis is strong the sphincter is more tightly contracted. In the earlier stages, when the gastric peristalsis is active, the muscle of the sphincter contracts tightly, as peristalsis grows more feeble the contractions of the sphincter become less intense until during the later stages of digestion, it is greatly relaxed as shown by the comparatively large lumen. During the systole of every gastric cycle, according to Cole the pylorus is open and through its lumen a small amount of liquid chyme is propelled into the reservoir cap. The terminal peristaltic contraction which has meanwhile been advancing over the antrum, upon attaining the sphincter effects its closure, so that the lumen is entirely obliterated or visible as a small line. Our radiograms upon the dog, see Fig. 7, are similar to those published by Cole. However, our graphic results show that the sphincter enters upon its positive phase sometime after the antrum has begun to contract and reaches its height only after the antrum is well along on its phase of relaxation. Further, our tracings show that not only the positive phase of the sphincter is influenced because of the propagation of a contraction wave into it from the antrum but also by the wave of relaxation which follows the contracting wave. This wave of necessity appears at the sphincter after having traveled the length of the antrum, that is, the sphincter demonstrates a phase of relaxation which is the continuation of the relaxation wave of the antrum. Hence, in a moderately active stomach a phase of inactivity is common to the antrum and sphincter: the sphincter remaining open or relaxed save for the degree of residual tone until such a time as its fibers are excited to contraction because of the approach of a contraction wave through the antrum.

The tracings shown in the work of Luckhardt, Phillips and Carlson<sup>12</sup> are corroborative of this last statement. These tracings clearly show that during an increase in tone of the stomach or at the approach of a powerful advancing ring of contraction material is forced from the stomach in ever increasing amounts, the final rush of material occurring with the termination of the peristaltic wave, and just prior to closure of the sphincter.

The motility in the antrum may be termed a compression wave which, from its origin at the proximal end of the antrum, by a process of constriction gradually presses down upon its contents. This constriction involves progressively rings of circular muscle nearer and nearer the sphincter and passes over the antrum at such a rate that the circular muscle next to the sphincter goes into a state of strong contraction before those at the proximal end have begun to relax. In other words, the advancing edge of the cycle in the antrum proceeds from above downward in the form of a wave but the duration of the constriction in the individual ring of circular muscle is so long that the entire antrum is in a state of contraction at the same time. Relaxation in the antrum follows the same order, namely, it appears first in the proximal end and gradually progresses

toward the sphincter. This perhaps means that the time of contraction stated in terms of the individual muscle fiber is uniform throughout the antrum.

In relation to the pars pylorica this compression wave in its progress involves more and more of the functional units, until those of the sphincter are reached and brought into action. The wave is also characterized by the holding quality of fibers already in action while new units are entering upon their active phase. For this reason the entire antrum may be seen to hold itself in a state of complete contraction at a time when the sphincter is just beginning to enter upon its positive phase. Relaxation always begins in the portion which first went into action and passes progressively over the pars pylorica. Therefore, the sphincter is the last portion of the pars pylorica to go into action and also the last to relax during a cycle of this portion of the stomach.

The above considerations lead to the assumption that the sphincter is opened during its quiescent phase (inhibition), either because of the pressure exerted upon it, by reason of an increased pressure in the antrum, which is associated with an increased degree of tonicity of the stomach as a whole, or because of a wave of inhibition continued from the antrum. This latter seems the more justifiable for the reason that the positive and negative phases of the antrum and sphincter are so placed that they cause the material in the stomach to be thrown forward. Also the manner of passage of the material through the sphincter is associated with the positive phase of the antrum and ceases when the sphincter contracts. Therefore, in regard to the stomach the sphincter demonstrates the "law of the intestine," i.e., it opens or is inhibited during the passage of materials and closes (contracts) when the advancing ring of contraction reaches it through the antrum.

#### SUMMARY AND CONCLUSION

To recapitulate, our results may be summarized as follows: Direct, graphic and radiographic observations show that the antrum and pyloric sphincters are rhythmical in action, that is, contractions and relaxations follow each other in a regular sequence. These rhythmical actions or cycles occur at the rate of from 3 to 5 per minute, and are maintained for hours. Rhythmical motility is best obtained when the stomach contains food, however, typical results may be obtained from the stomach recently emptied. Starved animals were not studied. The phases of activity in the sphincter are such as to supplement those of the antrum, hence, the motility of these two parts may be considered as constituting a cycle of the pars pylorica.

The sequence of action in the pars pylorica is as follows: During the active phase of the preantrum there occurs a loss of tone or inhibition in the antrum as a whole. This is immediately followed by a gradual or running contraction of the antrum, the entire antrum demonstrating a sustained contraction or plateau as the final act of its positive phase. This is followed by a wave of relaxation, which, starting in the preantral region, passes over the entire antrum. The sphincter, on the other hand demonstrates a negative phase (relaxation) at the time of beginning antral contractions. Some time before the antrum enters upon its held contraction the sphincter begins to rapidly contract and reaches its maximum, usually at a time of marked relaxation of the antrum. The sphincter

then relaxes and remains quiet until a second positive phase is well initiated in the antrum. Hence, the sphincter is open during the greater part of the antral contraction and actively closed while the antrum is relaxing.

Our results along with those of Cole and Luckhardt, Phillips and Carlson demonstrate that the activities of the pyloric sphincter, at least in great part, are dependent upon the activities of the antrum. That is, the impulse to contract in the antrum during digestive processes is propagated into the sphincter, thereby causing it to contract at a time of relaxation of the antrum, and to relax because of the arrival of a wave of relaxation over the antrum. Such conditions indirectly lead to the conclusion that acid if it acts to regulate the "pylorus" must also act in a similar way upon the antrum and stomach as a whole, for, as shown above, motility of the antrum determines motility of the sphincter.

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# LABORATORY METHODS

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## OBSERVATIONS ON THE QUANTITATIVE NATURE OF COMPLEMENT FIXATION

WITH SPECIAL REFERENCE TO THE CLINICAL APPLICATION OF PARTIAL  
WASSERMANN REACTIONS

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**B**OTH laboratory workers and clinicians have long felt the need of a standard technic for Wassermann tests. The experiments which Kolmer<sup>1</sup> and his coworkers are now carrying on will clear up many points regarding this test that have been obscure, and settle authoritatively many questions that have been in dispute. Their work will remove some of the difficulties which have heretofore stood in the way of a standardized technic. A finally acceptable test, however, must be based not on the work and opinions of any one man or any one set of men, but on the work and opinions and experiences of many men, both laboratory workers and clinicians. The wider the range of experience from which the details of the finally proposed test shall have been gathered, the greater the probability of its general acceptance. It is desirable, therefore, that as many as possible of those interested in this matter shall contribute their opinions and observations, and it is with this in mind that the present paper is offered.

It would be an easy matter to devise a technic so conservative that it would give positive reactions only with certainly syphilitic serums. But this technic would probably fail to detect many serums containing only small amounts of fixing substances. On the other hand, it would be an easy matter to devise a technic which would detect specific fixing substances in every syphilitic serum, no matter how small the amount; but this method would probably give inhibition with some negative serums. The problem is to devise a method that will give positive reactions, even partially positive reactions, only in the presence of syphilis, and negative reactions always in the absence of syphilis.

In all cases of fully developed secondary, and, in many cases of tertiary syphilis, the serums contain so large an amount of complement fixing substances that they will always be detected by the classical test even with an indifferent technic, and in such cases there is never any variation in the findings of different workers unless some very gross error has been committed. Nor will this method, when performed with reasonable care, ever give a complete reaction with a nonsyphilitic serum. It is in those cases of primary and latent or slightly active tertiary syphilis, in which only small amounts of complement fixing substances are present in the serum, that variations occur in the results obtained with different methods and even with the same methods employed by different workers. It is for the detection and standard evaluation

of these cases, which fall in or near the zone of partial reactions, that the need for a uniform standard test is chiefly felt.

Before undertaking the modification of the Wassermann test for purposes of standardization, it should be definitely determined whether the present classical method tends to err too much on the positive side, giving partially positive reactions in negative cases; whether it tends to err too much on the negative side, giving too many negatives with syphilitic serums, or whether it errs on both sides, giving partial positive reactions sometimes in nonsyphilitics and negative in cases that should give positive reactions.

The general opinion of most clinicians and of most laboratory workers is that partial reactions, especially mildly positive reactions obtained with the present classical test, are of doubtful value. The statement "one-plus or two-plus Wassermann reactions mean nothing" has been so frequently repeated that it has come to be almost axiomatic. At most these reactions are considered only of corroborative value in cases which present indefinite history or symptoms. I have been unable to find any experimental evidence in the medical literature that would give validity to this generally accepted interpretation of partial reactions. I believe the attitude of the clinicians regarding partial reactions is largely due to the fact that the performance of the Wassermann test has been and still is often left to incompetent and insufficiently trained assistants, who commit gross technical errors. Fully as necessary as a standardized technic is a standardization of the qualifications of those who are permitted to perform the test, and provisions for enforcing recognition of these standard qualifications on all laboratories.

I am of the opinion that the classical Wassermann test, when performed with reasonable care by a skilled technician, will never give partially positive reactions in any but syphilitic cases, and this opinion is based on the following considerations:

(a) The originators of the Wassermann test attempted to devise the technic with a very large factor of safety against the occurrence of false positive reactions. They knew that all serums have an anticomplementary action, and that this action varies considerably with different serums. The watery extract of syphilitic liver originally used for antigen, but now almost entirely discarded, was subject to vagaries, deteriorated rapidly, and was very apt to give false, proteotropic reactions. To overcome the possibility of false positive reactions as a result of these factors, an additional unit of complement was allowed in the original Wassermann technic. This ample allowance was made because the exact extent to which the above sources of error (and perhaps some unknown sources of error) might act was not known, and it was imperative that if the test erred at all it be on the negative and not on the positive side.

It is also possible that this large extra allowance of complement was found necessary because the possibility of error due to variations in the complement content of guinea pig serums was not recognized and provided for. The preliminary titration of the original technic did not standardize the complement dose, but provided for the compensation of a deficiency of complement by the

use of an excess of amboceptor. It is now well known that this is not a valid procedure, as it does not keep the complement dose in the second part of the test at a uniform level, and may, therefore, result in false positive reactions.

Every serologist worthy of the name now guards against this possible source of error either by making a preliminary titration of complement with a standard amboceptor and corpuscle suspension, or by pooling the serums of a number of guinea pigs, or still better, by both procedures. When this precaution has been observed, keeping the complement unit at a uniform level, it will be found that for fresh or carefully preserved serums, obtained from blood withdrawn under proper conditions, the extra unit of complement allowed for anticomplementary action of serums is four or five times the amount really required for this purpose. Furthermore, the alcoholic extracts of normal organs now almost universally used as antigens in place of the original watery extracts of syphilitic livers are very stable, and if properly prepared will give ample antigenic action in doses that exhibit only negligible anticomplementary properties. The classical test, therefore, as at present performed, refined to eliminate the grosser errors of the original technic, yet retaining the device of using an additional unit of complement originally resorted to for overcoming these gross errors, tends to err altogether on the negative side.

(b) The difference between partial reactions and complete reactions near the partial zone is very little, if we take into consideration the amount of fixing substance present in the average serum from a case of fully developed secondary syphilis (which is considered a type of positive serum, and is recommended for use in titrating antigen and for positive controls).

In many cases of secondary syphilis, and in some cases of tertiary syphilis, the serum contains sufficient complement fixing substances so that one hundredth the usual Wassermann dose will give complete fixation with the

TABLE I  
TITRATION OF AVERAGE SERUM\*

Amount of positive serum	0.001	0.005	0.0075	0.01	0.05	0.1	0.2
Per cent of inhibition	5	30	60	100	100	100	100

\*Inhibition estimates were made by the author's method, a description of which will be published in the near future. All work was done with one-fourth Wassermann quantities, but is reported in full Wassermann quantities.

usual dose (two units) of complement. It is seldom that, in a case of fully developed secondary syphilis, one tenth the usual dose will not give complete fixation, and it is my experience that the average is about one twentieth. In Table I is shown a titration of such an average serum. It will be seen that 0.01 c.c. (5 per cent of the usual dose) still gives complete inhibition, while 0.0075 c.c. (2.75 per cent of the usual dose) gives only 60 per cent inhibition. This indicates that a difference of 2.25 per cent of the total fixing substance found in the average serum of secondary syphilis represents the difference between a complete and a partial reaction; in other words, the difference between a surely positive and a doubtful reaction. Many of the serums in tertiary syphilis that give complete reactions are only slightly above the zone of partial reactions, that is, contain just slightly more than 5 per cent of the

amount of fixing substance found in a typical, average positive serum. If such a serum can be considered definitely specific, then a serum containing only 1 or 2 per cent less fixing substance should also be considered specific, even though it falls within the zone of partial reactions.

(c) If we analyze a complement titration we shall find that the general conception that various degrees of specific reaction as reported for different tests represent definitely corresponding variations in complement fixation has no basis in fact. Table II shows the result of an incubation of increasing amounts of complement with constant amounts of corpuscles and amboceptor (practically a complement titration). It will be noted that 0.5 c.e. of complement gives just complete hemolysis which, therefore, is the unit; and twice this, or 1 c.e., the dose as used in the classical test. The first trace of inhibition is seen in the tube containing 0.4 c.e. complement, which means that 0.6

TABLE II  
RESULT OF AN INCUBATION OF INCREASING AMOUNTS OF COMPLEMENT WITH CONSTANT AMOUNTS OF CORPUSCLES AND AMBOCEPTOR

Amount of complement.....	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.5	0.6	0.8	1.0
Per cent of inhibition.....	90	60	30	20	10	5	2	0	0	0	0
Per cent of complement dose.....	10	15	20	25	30	35	40	50	60	80	100
Per cent of complement unit.....	20	30	40	50	60	70	80	100			

c.e. or over half of the total amount of complement employed in a test must be absorbed before a trace of inhibition is registered. The tube containing 0.2 c.e. of complement, corresponding to an absorption of 0.8 c.e. in a test, shows only 30 per cent inhibition, approximately a one-plus reaction. This means that 80 per cent of the complement dose employed in a test must be absorbed before a one plus reaction is registered. From this point on the rise is very rapid, the additional absorption of only 5 per cent more complement giving 60 per cent inhibition, and another 5 per cent giving 90 per cent inhibition. It is seen, then, that 80 per cent of the complement employed in the classical Wassermann test must be absorbed before the zone of partial reactions is reached, and the difference between a one-plus and a four-plus reaction is represented by the remaining 20 per cent of complement.

It is true, of course, that in the classical test only half of the complement is meant for specific action. It has already been shown, however, that with the refinements which have been introduced in the classical test the absorption due to anticomplementary action is only a small fraction of the additional complement unit allowed for this purpose. But even if this entire unit of complement were absorbed nonspecifically, it would still be found that 60 per cent of the complement unit left available for the specific reaction must be absorbed before a one-plus reaction is registered; that 10 per cent further fixation gives two-plus reaction and 10 per cent more brings it between a three- and four-plus reaction.

That these facts are not in accord with the great difference in clinical value generally given to partial and complete reactions is apparent. They further corroborate my contention that there is very little difference between partial reactions and complete reactions bordering on the partial zone.

(d) There seems to be a general misunderstanding regarding the effect on hemolysis of variations in the quantitative relations between complement and fixing substance. The usual conception is that hemolysis (or inhibition) in the indicator system varies in direct proportion to the variations in the quantitative relations of the complement and fixing substance in the first part of the test, assuming the antigen to be constant. It is thought, for instance (and theoretically this should be true), that a serum which will give just slightly less than complete inhibition with one unit of complement will give complete hemolysis with two units, because the additional unit should remain available for the indicator system and is sufficient to complete hemolysis. Practically, however, this is not true. Reference to Table III shows that a serum which gives only 75 per cent inhibition with 1 c.e. complement dilution still gives 40 per cent inhibition with 2 c.e. A serum which gives 50 per cent inhibition with 1 c.e. still gives 20 per cent with 2 c.e., and one which gives 25

TABLE III

VARIATIONS IN FIXATION WITH SPECIFIC SERUMS RESULTING FROM VARIATIONS IN COMPLEMENT QUANTITIES\*

Amount of complement.....	0.8	1.0	1.5	2.0
Per cent inhibition with one plus serum.....	30	25	15	5
Per cent inhibition with two plus serum.....	60	50	40	20
Per cent inhibition with three plus serum.....	85	75	60	40

\*Average findings with a number of serums.

per cent with 1 c.e. still gives 5 per cent with 2 c.e. It is apparent, then, that the relative amount of complement fixed in the presence of a constant amount of antigen and fixing substance increases as the volume of complement is increased or, vice versa, decreases as the volume is decreased. I have, furthermore, experimental evidence to show that this same law, if I may so call it, also operates in the case of nonspecific fixation. From these facts it will be seen that Wassermann tests are, in a measure, self-corrective as far as errors due to variations in complement quantities are concerned. Technicians know that, even with the greatest care, there will often be more or less variation in the complement quantities on different days. With the present conception of the quantitative nature of complement fixation, it is thought that such variations might at times bring about false positive reactions. However, in view of the real quantitative nature of complement fixation and its self-corrective action, which I have demonstrated, I believe it is safe to say that, when the usual ordinary precautions have been taken to keep the complement dose at a constant level, such differences as are unavoidable with the present technic will never be sufficient to raise a negative serum into the zone of partial reactions.

(e) We have, for a number of years, in our laboratory, tested all serums with a raw serum method in addition to the usual classical test. This raw serum method provides corrections of errors that may be due to variations in native complement content of raw serums, due to the presence of hemolysin homologous to that of the indicator system, and to variations in nonspecific fixation which are usually more pronounced with raw than with inactivated



serums. The method has, therefore, the advantage of increased delicacy due to the use of noninactivated serum, but is without the disadvantages usually found in other raw serum tests that tend to lead to false positive reactions. An analysis of the comparative findings which we have obtained in cases known to be syphilitic, and in presumably nonsyphilitic cases, supports my view that the classical test errs far too greatly on the side of falsely negative reactions, and that in practically all cases in which it enters the zone of partial reactions, these are specific in nature. In every case without exception in which the classical test gave a mildly positive reaction, the raw serum test gave a much more strongly positive reaction. With but few exceptions when inhibition with the classical test was as high as 50 per cent, the raw serum test gave complete inhibition. Not infrequently, in cases known to be specific, especially in cases under treatment, the classical test was completely negative when the raw serum test was partially positive and sometimes even completely positive.

(f) One of the reasons why the specificity of partial reactions has come to be doubted is that they have not infrequently been found in persons who give no history of syphilis and who present no symptoms of the disease. Complete reactions are also encountered in this class of individuals, but because of the misconception regarding the quantitative relation between partial and complete reactions, the latter have been considered sufficient to justify the classification of individuals on whom they have been obtained as definitely syphilitic, while the former have, erroneously enough, been considered as of no significance.

The value of a negative history seems to be overestimated. Many strongly positive and many partially positive reactions are obtained on persons giving definite symptoms of syphilis, who have no knowledge of the primary infection. Why, then, attach much significance to a negative history in the presence of partial reactions without history?

Furthermore, absence of symptoms cannot be considered a criterion of the absence of syphilis. The symptoms of syphilis, and especially tertiary syphilis, depend not so much on the character and extent of the lesions as on the location of the lesions. Lesions which in the cortical areas of the cerebrum, in the cord or iris would produce definitely recognizable symptoms, might be completely asymptomatic in the liver or spleen. In this connection the work of Warthin<sup>2</sup> who found evidence of syphilis in approximately 40 per cent of his necropsy material, is significant and cannot be ignored. Many of these cases presented no symptoms of syphilis during life, and gave negative Wassermann reactions. Our past conception of syphilis is probably a mistaken one. Undoubtedly the resistance of individuals to this disease varies just as much as it does to any other disease, with corresponding variations in the resultant manifestations. From the highly susceptible individual with little resistance, in whom the fulminating type develops, down to a favored few who are entirely immune, there may be every grade of susceptibility. There are probably many persons who become infected without showing any recognizable primary or secondary manifestation, and who carry the spirochetes for years in relatively unimportant tissues where they produce slowly productive lesions. The organisms may eventually become entirely dormant, or if the

resistance of the individual is lowered, as through intercurrent disease, exposure or senility, they may again become active and produce definite symptoms.

With this conception one can readily understand that partial reactions may be obtained on the blood of apparently healthy individuals, and that the mere fact that such reactions do occur cannot be considered valid evidence against their specificity.

The observations which I have presented herewith would indicate that the technic of the present classical test is extremely conservative, and provides a very wide margin of safety against the occurrence of false positive reactions. It would seem, also, that this margin of safety can be considerably narrowed without incurring the danger of false positive reactions, thereby increasing proportionately the efficiency and delicacy of the test.

Of course, if the partial reactions, which at present are looked on with suspicion, are accepted as unqualifiedly specific, that in itself will very materially increase the efficiency of the test. However, if a test can be devised that will make these partially positive reactions strongly or completely positive, a considerable advantage will be gained, because such reactions will have more conclusive value for both the physician and his patient. Furthermore, a more delicate test would give many strongly positive reactions in cases of syphilis that give completely negative reactions with the classical test. This is especially important for cases under treatment. It has, for instance, been my experience that, without a single exception, cases of syphilis under treatment have given positive reactions with raw serum tests long after the reactions with the classical test have become negative.

It is my opinion that any effort to standardize the Wassermann test will not be successful unless the technic adopted gives a very material increase in efficiency over the present method. I also believe that the highest possible degree of efficiency cannot be attained unless the method provides for testing the serum in the noninactivated state.

I realize that a great deal of prejudice exists against noninactivated or raw serum tests. This is largely due to the fact that practically all raw serum tests that have been proposed have been inexact, unscientific and subject to false positive reactions. But I am sure that a raw serum test can be devised which is exact and scientific, which will be more delicate than inactivated serum tests, and still provide an adequate factor of safety against false positive reactions.

For the present, perhaps, the ideal method would be one that includes a test with both raw and inactivated serums. This would enable us to retain our present standards of value for comparison with the raw serum method, and provide an easy stage of transition to the exclusive use of a raw serum method, should this later on be found to be justified.

The technic of the standard test should also be such that a definite, standard value can be given to partial reactions, so that there may be no confusion regarding the interpretations which are to be given them. This evaluation should be the result of careful and prolonged clinical observation, and, if possible, of postmortem study after the method of Warthin.

## METHOD OF TESTING SERUMS

In closing this paper, I will briefly describe the method of testing and the method of interpretation which have been adopted in my laboratory as a result of many years of experience and observation, not because I think it ideal, for I realize that there is much room for improvement, but in the hope that it contains some suggestions that may be of value to those who have engaged in the effort to standardize the Wassermann test.

All serums are tested with the classical method, modified to avoid as far as possible the major sources of error in the original Wassermann test as follows:

(a) Variations in the complement content of guinea-pig serum are overcome by using pooled serums and a preliminary titration with amboceptor of known strength and standard corpuscle suspension.

(b) Variations in the specific fixability of guinea-pig serums are corrected by the use of pooled serums.

(c) Errors due to variations in the anticomplementary action of different human serums are minimized by varying the second incubation period for each serum in accordance with the hemolyzing time of the serum controls.

(d) Variations in the sheep hemolysin content of different human serums are corrected by the method devised by me.<sup>2</sup>

(e) A standard antigen is used, the antigenic properties of which have been determined by titration with positive serums of known strength.

(f) A partially positive serum of known strength is used as a positive control in the test.

In addition all serums are tested with my raw serum method,<sup>4</sup> which guards against the following sources of error:

(a) Quantitative variations in the native complement content of human serums.

(b) Quantitative variations of hemolysin in human serums homologous to that employed in the indicator system.

(c) Variations in the anticomplementary properties of different serums. Findings are given the following interpretations:

**Definitely Specific:** All serums that give 100 per cent inhibition with the raw serum test, regardless of the findings with the classical test. All serums that give 50 per cent inhibition or over with the classical test, but less than 100 per cent with the raw serum test.

**Probably Specific:** All serums that give less than 50 per cent inhibition with the classical test, but more than 50 per cent and less than 100 per cent with the raw serum test.

**Doubtful:** All serums that give less than 50 per cent inhibition with the raw serum test.

**Negative:** All serums that give complete hemolysis with both tests.

For the practical application of these reactions, cases are grouped into four groups, as follows:

**Group I:** Cases that give an unmistakable history of syphilis, but with no symptoms, and who have or have not been under treatment. A single nega-

tive in these cases does not exclude syphilis. Negative reactions over a period of two years are considered evidence that the disease has been cured. Any partial reaction indicates a persistence of the disease.

Group II. (a) Cases that present unmistakable symptoms of syphilis. (b) Cases with obscure symptoms, but which have been diagnosed by the specialist (internist, neurologist or dermatologist) as syphilitic. In this group the Wassermann test is not essential, but is made for corroborative purposes or to convince the patient. Treatment is undertaken regardless of the outcome of the test.

Group III. Cases with no definite history of syphilis, and with symptoms which cannot be definitely referable to the disease, but which may nevertheless be caused by it. "Definitely Specific" reactions in these cases indicate that syphilis is present, and that the symptoms are probably, though not necessarily, caused by it.

"Probably Specific" and "Doubtful" reactions justify, in my opinion, the institution of antisyphilitic treatment, though conservatism demands that a frank diagnosis of syphilis be withheld until the results of the treatment are evident. If definite improvement results, the diagnosis is made and the case treated according to individual requirements. If no improvement results, a diagnosis of syphilis cannot be made. These reactions, then, are considered merely justification for resorting to the therapeutic test. In order to avoid unnecessary anguish of mind it is desirable, if possible, to withhold from the patient any information as to the nature of the disease suspected until the diagnosis has been confirmed by the therapeutic test. In these days of needle therapy, it should not be difficult to give intramuscular injections of mercury, or even intravenous injections of arsphenamine without divulging the true nature of the treatment.

In cases in which symptoms point to a nervous involvement, a spinal puncture should, of course, be made.

Group IV. Cases with no history of syphilis, and with no symptoms of the disease, in whom the test is made in the course of routine examinations, or for experimental, statistical or eugenic purposes. A "Definitely Specific" reaction in any of these cases means syphilis, without question. A "Probably Specific" reaction is highly suspicious. Whether such persons should be placed under treatment, or merely kept under observation, is a question to which there are two sides and to the discussion of which I cannot give space in this paper. A "Doubtful" reaction should probably be disregarded in this group.

#### SUMMARY

There is urgent need for a standard technic for Wassermann tests. The need of standard requirements for technicians is just as urgent.

The classical test, when properly performed by a skilled technician, tends to err altogether on the negative side. Partial reactions are always specific.

The aim should be to make the standard test more delicate than the present classical test, without sacrificing its specificity, so that a larger number of

definitely positive reactions may be obtained in cases of syphilis in which the blood contains only small amounts of fixing substances.

It is suggested that this be accomplished by testing all serums with the classical method modified to remove its major sources of error, and a raw serum test modified to remove the usual sources of error inherent in present raw serum methods.

A scheme of interpretation of partial reactions and their clinical application is given. This gives these reactions a more definite clinical value than they now have.

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## COMPLEMENT VS. AMBOCEPTOR TITRATIONS IN THE WASSERMANN TEST\*

BY R. L. KAHN, SC.D., LANSING, MICH.

THIS paper will endeavor to prove the desirability (1) of standardizing the time of incubation of amboceptor and complement titrations and the time of final incubation before reading the tests, to a 15 minute period and (2) of titrating both complement and amboceptor daily in the Wassermann test (guinea-pig complement, sheep-cell system).

1. The Wassermann test may be said to consist of the following two steps: First, the finding of the smallest amount of complement necessary to bring about hemolysis of a standard amount of red cells in the presence of a standard amount of amboceptor. Second, the ascertaining whether this (or a multiple of this) amount of complement will hemolyze the red cells in the presence of amboceptor, unknown serum and Wassermann antigen; or whether the latter (serum and antigen) will "fix" the complement and thereby prevent hemolysis of the cells.

Viewing the Wassermann test from this angle, it is evident that the physical factors which influence hemolysis of red cells, such as the nature and period of incubation, ought to be the same in the first step of the test as in the second. However, while most workers resort to the water-bath as the mode of incubation, they do not always employ the same period of incubation in both cases. To illustrate: one worker might employ a half-hour incubation period in his complement titration, (first step of test) and one hour incubation period before reading his tests (second step of test); while another might employ an incu-

\*From the Bureau of Laboratories, Michigan Department of Health, Lansing, Michigan.

bation period of one hour in his complement titrations and approximately fifteen minutes incubation period before reading his tests.

One frequently finds still another variable element in the hemolytic system of the Wassermann test, namely, the time of incubation of amboceptor titrations. It is difficult to see why the incubation period of amboceptor titrations should vary from the incubation period of complement titrations in any given procedure; and it is no less difficult to see why the incubation periods of both of these titrations should differ from the final incubation period before reading the tests. Yet, as is illustrated by the table given below, these variations are not uncommon, Kolmer being the only worker listed, who employs a constant incubation period throughout.

TABLE I

ILLUSTRATING VARIATIONS IN THE LENGTH OF INCUBATION PERIODS OF AMBOCEPTOR AND COMPLEMENT TITRATIONS AND OF FINAL TESTS, AS EMPLOYED BY DIFFERENT WORKERS

NO.	AUTHOR	LENGTH OF INCUBATION PERIOD OF AMBOCEPTOR TITRATIONS	LENGTH OF INCUBATION PERIOD OF COMPLEMENT TITRATIONS	LENGTH OF FINAL INCUBATION PERIOD, BEFORE READING TESTS
I	Hinton <sup>1</sup> (Mass. State Board of Health)	1 hour	$\frac{1}{2}$ hour	1 hour
II	Kolmer <sup>2</sup>	1 hour	1 hour	1 hour
III	Koopman <sup>3</sup> (N. Y. City Board of Health)	1 hour	1 hour	Approx. 15 min. (Tests are read as soon as controls hemolyze)
IV	Neill <sup>4</sup> (U. S. Public Health Service)	1 hour	$\frac{1}{2}$ hour	$\frac{1}{2}$ hour
V	Simon <sup>5</sup>	$\frac{1}{2}$ hour	—	10-15 minutes
IV	Thomas and Ivy <sup>6</sup>	1 hour	1 hour	1.5 to 2 hours

Granting the importance of employing a standard incubation period for the hemolytic phase of the Wassermann test, it was felt that the adoption of a 15-minute period would serve the purpose best, since it would practically eliminate the old-disputed question whether to titrate complement or amboceptor in this test. Those workers who employ amboceptor instead of complement titrations in the Wassermann test, do so largely, not because amboceptor titrations render the Wassermann results more accurate, but rather because these titrations render the procedure of the test, more practical. Daily complement titrations necessarily delay the completion of the Wassermann tests, while amboceptor titrations do not and are preferred for this reason. By adopting, therefore, a 15-minute incubation period, the delay produced by complement titrations becomes comparatively insignificant, and the time objection against these titrations, is practically eliminated.

A solution to this problem was attempted by studying the amount of hemolysis produced in amboceptor and complement titrations during 15-minute, 30-minute and 1-hour incubation periods.

To begin with, the unit of new amboceptor was determined by preparing a series of dilutions of amboceptor serum and titrating each with 0.1 c.c. of a 5 per cent suspension of sheep cells and 0.1 c.c. of 1:10 pooled guinea pig complement (one-tenth quantities of

classical Wassermann). The titrations were carried out in a series of 10 tubes, in the following proportions:

Tube	1	2	3	4	5	6	7	8	9	10
Amboceptor (c.c.)	.1	.09	.08	.07	.06	.05	.04	.03	.02	.01
Complement (c.c.) (1-10)	.1	.1	.1	.1	.1	.1	.1	.1	.1	.1
Sheep-Cells (c.c.) (5%)	.1	.1	.1	.1	.1	.1	.1	.1	.1	.1
Saline (drops)	2	2	2	3	3	3	3	4	4	4

The unit was read in each case after 15 minutes incubation in the water-bath. For the sake of simplicity all the reagents in the Wassermann test are employed in 0.1 c.c. quantities. The unit aimed at, therefore, was 0.05 c.c., so that 0.1 c.c. would contain 2 units of amboceptor.

After finding the dilution of amboceptor serum which gave a unit of 0.05 c.c., the titrations were repeated daily for a week with different pooled complements in order to establish a permanent amboceptor unit.

The amboceptor unit being determined, complement units were obtained by employing pooled 1-10 complement in quantities ranging from 0.1 to 0.01 c.c., with 2 units of amboceptor and 0.1 c.c. sheep-cells, after incubation for 15 minutes in water-bath.

It was soon found that with the procedure employed in this laboratory, the difference between 15 minutes and  $\frac{1}{2}$  hour incubation was frequently so slight as to make it too indefinite for accurate readings and that the difference between 15 minutes and 1 hour incubation, was nothing more than one gradation in either complement or amboceptor titrations. Thus, if the complement or amboceptor unit was 0.05 at the end of 15 minutes incubation, it was 0.04 at the end of one hour incubation and if the unit was 0.04 at the end of 15 minutes it was 0.03 at the end of an hour.

By employing a 15 minute incubation period in our tests, it was a comparatively simple matter to observe the differences in these units. Thus, after reading the respective units after 15 minutes incubation, the titration racks were merely permitted to remain in the water-bath for 15 minutes longer and the units determined again. These readings were carried out daily for several months as a matter of routine, without however, keeping a record of them.

Table II based on 10 recent daily titrations, illustrates the marked constancy in the difference between 15 minutes and 1 hour incubation. The complement titrations are regularly being carried out in duplication in this laboratory because of the importance of employing a proper amount of this ingredient.

Ottenberg,<sup>7</sup> in discussing the incubation period of complement and amboceptor titrations, states that 1 hour is too long for practical work and 15 minutes probably too short, and suggests the adoption of a 30-minute period. According to this worker, "The 15-minute period is probably too short to allow for slight differences in the speed with which the mixtures assume the temperature of the thermostat, due to variations in the thickness of the glassware, etc." We have been employing Wassermann tubes ranging in thickness from 0.025 to 0.075 of an inch, without detecting after 15-minute incubation any variations in our complement and amboceptor titrations due to these differences in thickness. Neither have we been able to detect any other factors which tend to interfere with the correct determination of these units after this short period of incubation.\*

A distinct advantage in the employment of a 15-minute incubation period.

\*The writer is informed that Dr. Ottenberg has been for some time employing a 15-minute incubation period; and that Dr. Kaliski also, at the laboratory of the Mt. Sinai Hospital, New York, has been employing this short incubation period.

TABLE II

SHOWING THE EFFECT OF 15 MINUTES AS COMPARED WITH 1 HOUR INCUBATION ON THE DETERMINATION OF THE UNIT IN COMPLEMENT AND AMBOCEPTOR TITRATIONS

DATE 1920 Sept.	UNIT OF COMPLEMENT AFTER		UNIT OF AMBOCEPTOR AFTER	
	15 Minutes Incubation c.c.	1 Hour Incubation c.c.	15 Minutes Incubation c.c.	1 Hour Incubation c.c.
8	.05	.04	.05	.04
	.05	.04		
9	.04	.03	.05	.04
	.04	.03		
10	.03	.02	.04	.03
	.03	.02		
11	.04	.03	.04	.03
	.04	.03		
13	.05	.04	.05	.04
	.05	.04		
14	.04	.03	.05	.04
	.04	.03		
15	.05	.04	.05	.04
	.05	.04		
16	.04	.03	.05	.04
	.04	.03		
17	.04	.03	.05	.04
	.04	.03		
18	.05	.04	.06	.05
	.05	.04		

may be mentioned in this connection. When titrating pooled complement from 3 or 4 guinea pigs on any given day, it not infrequently happens that one of the sera is of poor complement potency and that it would be better not to use it in the mixture. With the employment of an incubation period lasting 1 hour, it is often difficult to detect the presence of the slow complement, since by the end of that time, the hemolysis is so nearly complete, due to the potent complements, that the poor complement is all covered up, so to speak. With the employment of a 15-minute incubation period, however, the finding of a slow complement in a pooled mixture is a comparatively simple matter. The series of titration tubes usually show about 90 instead of 100 per cent of hemolysis, indicating the presence of a serum which is "preventing" hemolysis. Under these conditions, each pig is titrated separately and the poor complement discarded.

In this laboratory a 15-minute incubation period is being employed for complement and amboceptor titrations, and approximately 15 minutes for the final incubation before reading the tests. Our experience with this procedure is based on 10,000 Wassermann tests. And in our opinion this short incubation period is highly desirable from both a theoretical and practical viewpoint.

2. The problem whether to titrate complement or amboceptor in the Wasser-



mann test, has recently been revived by Kolmer, Matsunami and Rule. After an extensive study of this question, these investigators make, among others, this significant statement: "Adjustment of the hemolytic system by daily titration of complement has proved superior to adjustment by daily titration of hemolysin for the conduct of complement-fixation tests of syphilis." Among other workers, Ottenberg particularly has been emphasizing for the past several years the importance of daily titrations of complement in preference to amboceptor in the Wassermann test. Most workers it appears, are at present agreed on this point, except that in laboratories where a large number of tests are performed daily, complement titrations are impractical because of the delay they cause in the completion of the tests. This is especially true in those cases where 1-hour incubation periods are employed.

With the reduction of the time of incubation of the hemolytic system to 15 minutes, the element of delay practically disappears. In this laboratory with approximately 100 Wassermann tests daily, we find this element to be quite negligible. It takes from 3 to 4 minutes to set up a complement titration and with 15 minutes incubation, the complement unit is usually obtained within 20 minutes.

We do not, however, stop with this titration. We daily titrate amboceptor as well. This is done not because of the slight changes in the keeping quality of the amboceptor, but in order to insure that no less than two units of amboceptor will be employed with the complement and cell suspension of any given day. And as a result of this extra titration we have been able to avoid the occasional difficulties which come up in a Wassermann laboratory, due to an unbalanced hemolytic system.

To begin with, we do not titrate the hemolytic system in the presence of antigen. Such titrations ought to include a fixation period of the same length of time and at the same temperature as carried out in the tests. Thus, in our procedure, the complement titrations with alcoholic extract antigen, ought to be given a four-hour fixation period in the ice box, since we employ this mode of fixation in our alcoholic-antigen tests. Neither do we titrate our complement in the presence of pooled negative sera, since the sera employed would necessarily have to be that left over from the tests—in other words, comparatively old—and as is well-known, the older the serum the more complement it absorbs; it is questionable therefore, whether the complement absorption of such pooled sera would represent the amount of complement absorbed by the comparatively fresher sera employed in the test. In our opinion, the titration of complement in the presence of amboceptor and cells and the employment of two units of complement, are, so far as our present knowledge goes, highly adaptable for a routine laboratory.

To return to the amboceptor titrations, it will be recalled that the original amboceptor unit is obtained by titrating different dilutions of amboceptor-serum with a series of pooled complements diluted 1-10. For convenience, this amboceptor unit is contained in 0.05 c.c. of diluted amboceptor so that 0.1 c.c. is equivalent to two units. Now, after titrating the complement on a given day with 0.1 c.c. amboceptor as the constant and determining the proper dilution which will contain two units, it not infrequently happens that if the amboceptor

is titrated back with the two units of complement as the constant, that 0.1 c.c. of amboceptor contains less than two units. To illustrate his point, we will consider the titration of the hemolytic system of September 18th, as recorded in Table II.

1. The complement consisted of fresh pooled serum from three guinea pigs diluted 1-10.  
2. The amboceptor was immune rabbit serum in a dilution of 1:2200, 0.1 c.c. of which contained two units.

3. The sheep-cells were obtained on the day before, from our own sheep, washed four times with saline and centrifuged at 1600 r. p. m. for 14 minutes, after which they were made up into a 5 per cent suspension.

4. Complement titrations were carried out with quantities ranging from 0.1 c.c. to 0.01 c.c. employing 0.1 c.c. amboceptor (2 units) and 0.1 c.c. sheep-cells, as constants. These titrations were carried out independently by two workers and the unit obtained in each case after 15 minutes incubation in water-bath, was 0.05 c.c. Therefore, 0.1 c.c. of 1-10 complement was used in the tests.

5. An amboceptor titration was then carried out several hours later (during the fixation period) and the unit after 15 minutes incubation, was found to be 0.06 instead of 0.05 c.c. If therefore, we had used 0.1 c.c. of amboceptor in our final tests, we would have employed less than two units of amboceptor.

6. In order to bring the amboceptor dilution to a point where two units will be contained in 0.1 c.c., the following proportion was resorted to:

$$\begin{aligned} 2200 : 06 :: x : .05 \\ x = 1833 \end{aligned}$$

Based on this finding, the amboceptor was diluted 1:1800 and 0.1 c.c. used in the final tests. In this way an unbalanced hemolytic system was avoided on that day.

The fact that the potency of amboceptor is comparatively constant for long periods has led most workers to believe that once the amboceptor unit is established it is not necessary to titrate it every day; that the daily titration of complement is sufficient. It is questionable, in our opinion, whether this reasoning is correct. It is not the titre but the keeping quality of amboceptor that is constant. The titre varies daily with the potency of the complement and with the slight changes in resistance and concentration of the sheep-cell suspension. A glance at Table II, shows a tendency for the daily amboceptor titration to run somewhat weaker than the corresponding complement titration on certain days. This is undoubtedly due to the fact that while complement titrations are generally carried out the first thing in the morning with complement just removed from the ice box, amboceptor titrations are carried out several hours later—after the Wassermann sets are undergoing fixation in the ice box or water-bath. During this interval the complement would naturally undergo slight deterioration. It is not unlikely that although the original amount of complement was two units, that by the time the amboceptor titration is carried out,  $\frac{1}{8}$  to  $\frac{1}{4}$  of a unit has deteriorated. If to this is added the changes in the resistance of sheep-cells, it is easy to understand, why the amboceptor titre is frequently less than two units.

To state this in another way, two units (instead of one unit) of complement are employed in the Wassermann tests in order to overcome the nonspecific binding of serum and antigen, but possibly not enough provisions are made for the natural deterioration of complement during the fixation period. We start out with two units of complement in our tests and by the time cells and amboceptor

are ready to be added, we might be dealing with  $13_4$  complement units instead of 2. Observations carried out in this laboratory, lead us to believe that this deterioration element is of particular importance if a given pooled complement contains a serum of low complement potency. Our findings indicate that the poorer the complement, the faster it deteriorates; and when starting out with two units of pooled complement, it is not unlikely that due to one serum of low potency, the titre of the mixture at the end of the fixation period, might be considerably less than two units. It is evident, therefore, that an amboceptor titration during the fixation period tells us whether the complement has undergone sufficient deterioration to affect the balance of the hemolytic system. If the amboceptor titration is somewhere above two units, we make no change, but if below two units, we bring it up to two units according to the procedure outlined above.

It takes no more than 20 minutes to complete an amboceptor titration. Furthermore, it is carried out during a period when Wassermann workers have ample time; no practical objections, therefore, can be raised against it. The advantage derived from these titrations, however, is quite important. The employment of a properly balanced hemolytic system from day to day, forms the basis of correct Wassermann tests. In this laboratory, aside from the employment of a 4- and negative serum control, a doubtful control also, is included in the daily tests. A doubtful test is somewhere between a 1- and a negative; it is a serum showing in the neighborhood of 12.5 per cent complement binding. These doubtful controls practically check from day to day, and in our opinion, this is due to the fine graduation of the hemolytic system, resulting from the combined complement and amboceptor titrations. Furthermore, we employ a cholesterinized antigen of pig's hearts with water-bath fixation and an alcoholic-extract antigen of beef heart with ice box fixation, and our tests check nearly 100 per cent. Our records show that in the last 8000 tests, only 1 in 1500 did not check.\* This also we believe, is largely due to the combined complement and amboceptor titrations.

#### CONCLUSIONS

1. The time of incubation of amboceptor and complement titrations and the time of final incubation before reading the tests, should be the same in any given procedure; the standardization of this incubation period of 15 minutes, is highly desirable.
2. The daily titration of both complement and amboceptor in the Wassermann test, is necessary to a properly balanced hemolytic system.

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\*We consider a strong positive by one method and a weak positive by the other, a check; while a strong or weak positive by one method and negative by the other, is repeated as a matter of routine.

# A COMPOSITE REAGENT FOR THE DETERMINATION OF SODIUM CHLORIDE IN URINE\*

By H. V. ATKINSON, CHICAGO, ILL.

**I**N the determination of sodium chloride in urine, it has been found very convenient to make up the following solution:

Silver nitrate	6.67 Grams
Ferrie ammonium alum	75 Grams
Nitric acid, conc.	150 c.c.
Water to	1000 c.c.

There is also needed the usual ammonium thiocyanate solution, 1 c.c. of which equals 0.01 gm. of sodium chloride, which can be made overstrength by dissolving 14 gm. of ammonium thiocyanate in 1000 c.c. of water and checking against the above reagent, 4.5 c.c. of which should equal 1 c.c. of ammonium thiocyanate solution; water is added to this solution until 4.5 c.c. of the composited reagent exactly equals 1 c.c. of the thiocyanate. The determination is made as follows:

Place 90 c.c. of the reagent in a glass stoppered 100 c.c. cylinder and add 10 c.c. of urine from a pipet. Shake and allow to stand for a few minutes, then pipet off 50 c.c. of the approximately clear solution and titrate the excess silver nitrate with the standard thiocyanate.

Calculation: Since 90 c.c. of the reagents equals 20 c.c. of the standard thiocyanate and only half of the excess silver nitrate is titrated  $20 - 2$  (20 - titration)  $\times 0.01 =$  gm. NaCl in 10 c.c. of urine used.

This reagent avoids measuring separately the water, nitric acid and saturated ferrie ammonium alum. The precipitate settles rapidly and renders filtration unnecessary. The absolute error of measuring 90 c.c. in a cylinder is no greater than the error of measuring 20 c.c. from a pipet. The use of two extra pipets can be avoided by using a very tall, accurately graduated, 100 c.c. cylinder and thus measuring the 10 c.c. of urine on top of the 90 c.c. of standard silver solution and likewise the 50 c.c. of clear supernatant liquid for titration.

\*From the Laboratory of Pharmacology, College of Medicine, University of Illinois, Chicago, Ill.

## THE UTILIZATION OF THE CILIARY GANGLION FOR CLASS WORK IN THE PHYSIOLOGY AND PHARMACOLOGY OF THE EYE\*

BY HUGH McGRIGAN, PH.D., M.D., CHICAGO, ILL.

**I**N the physiology and pharmacology of the eye, stimulation of the sympathetic nerve, as is well known, gives a marked dilation of the pupil. Constriction of the pupil is usually not demonstrated, except by varying the focus, or by the use of drugs.

Direct stimulation of the ciliary ganglion can be utilized almost as easily as stimulation of the sympathetic; and alternate stimulation of the two, makes the result of each more striking. An experiment conducted as follows is illustrative:

Anesthetize a dog, and isolate the vagosympathetic nerve; ligate and cut this. Use the central end for stimulation. Note the effect on the pupil. Now dissect in the temporal region sufficiently to expose the orbit to its depth. Find the external rectus muscle and dissect back carefully as far as possible. The ciliary ganglion is found internal to and under the external rectus, and under the superior rectus, close to the optic nerve and about an inch and one-half back of the eyeball (the distance depending on the size of the dog). The diagrams given in human anatomy locate this satisfactorily, and suffice as guides in the dissection. The ganglion is smaller than a pin head but plainly visible, and stimulation of it with a weak current leaves no doubt of its identity. It is advisable to cut the extrinsic muscles of the eye to prevent movement. It is advisable also to first dissect out the ganglion in a dead animal, to get its exact location.

We have found in most cases it is simpler to enucleate the eye with its extrinsic muscles and to work with it in warm (40° C.) saline. Such a preparation is usable for at least 15 minutes after removal. By stimulation around the cut end of the optic nerve in such a preparation, one can readily find dilator fibers, (long ciliary?) a little peripheral to this and to the temporal side the ciliary ganglion may be found, stimulation of which gives the greatest possible constriction of the pupil. We have already recorded the use of this in research work (*Jour. Pharmacol.*, July, 1920, xv, No. 5, p. 415) and use it in class room work. The action of nicotine and other drugs can be easily demonstrated on this ganglion.

A little practice in the dissection develops adequate skill in this striking and valuable experiment. Care is necessary not to stretch or tear the post ciliary fibers (short ciliary nerves).

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\*From the Laboratory of Pharmacology, College of Medicine, University of Illinois, Chicago.

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## EDITORIALS

### *Tuberculosis and Reinfection*

OF GREAT interest to workers in the tuberculosis field are two recent and excellent works, "L'Infection Bacillaire et la Tuberculose," by A. Calmette, and "A Study in the Epidemiology of Tuberculosis" by Col. G. E. Bushnell. The former is written by one of the greatest French investigators in tuberculosis, the latter by one of unusual clinical and pathologic experience and a master of the literature of this disease. Many parts of these works are worthy of discussion and may be referred to in these columns later; for the present the important question of reinfection will be considered.

In 1886 Marfan<sup>1</sup> announced the following "law" in tuberculosis: "One almost never finds pulmonary tuberculosis, at least manifest and a progressing disease, in people who in infancy have been the subjects of scrofula (suppurative tuberculous adenitis of the neck) and who have been completely cured of this before the age of fifteen, such cure having taken place before any other focus of tuberculosis was discoverable."

In 1891 Koch<sup>2</sup> announced the experiments in re inoculating tuberculous guinea pigs, which led him to introduce tuberculin. Briefly, these experiments showed a more violent local tissue reaction, together with greater

tendency to healing, in second inoculations than in primary ones. Later work on the same point has shown that healing of the second ulcer occurs only if the second dose of bacilli is fairly small. In the course of some experiments on inoculation by implanting tuberculous lymph nodes, in my laboratory, we have found that the second plant results in repeated cycles of ulceration and healing, as the bacilli are set free in caseous pus by necrosis, accumulate, break through the surface, and are discharged, while the ulcer resulting from the first plant never heals. In spite of the heightened local immunity in all these experiments, the disease progresses and the animals die.

In 1907<sup>2</sup> Calmette published the results of experiments with cattle, and claimed that without doubt tuberculous animals were incomparably more resistant to an intravenous test inoculation than normal animals. In 1908 he addressed a letter to practicing physicians in France in an effort to ascertain the truth of Marfan's "law." He wrote, "The experiences of the laboratory show that cattle nearly always recover from a single infection (by the digestive route) if carefully isolated, whereas they rarely recover, but become actively tuberculous, if they are infected several times at short intervals, *or if they are left in prolonged contact with tuberculous animals*. It appears then that cattle, just as man, cured of former lesions, may be in some manner 'vaccinated.' It is important that we should know if child and adult immunity against tuberculosis can be established following an old light or massive infection."

The answers received all agreed that severe forms of pulmonary tuberculosis were rare in people who had received in childhood a localized tuberculous lesion.

Calmette relates many experiences of different workers bearing on the Koch phenomenon. Römer's work of 1909 is quoted, which leads to the conclusion that "Tuberculosis of reinfection invariably takes the gait of a chronic infection." Bezançon and Serbonnes<sup>4</sup> showed that with the guinea pig early reinfection of a tuberculous animal—from the first to the fifteenth day after the primary infection—leads to abscess formation without healing. Only after the sixteenth to the eighteenth day does the phenomenon of Koch characterized by necrosis and healing take place. These time relations, by the way, correspond pretty closely to those governing the first appearance of the positive tuberculin reaction after experimental inoculation.

Grysez and Petit-Dutaillis<sup>5</sup> sought to ascertain the result of repeated inhalations at varying intervals of time in guinea pigs. They learned that many infections by inhalation in close succession were infinitely less dangerous to the animal than a single infection. If the reinfections were attempted late after the first infection, the Koch phenomenon followed and voluminous necrotic lesions with widespread tissue destruction developed. Bruyant<sup>6</sup> injected guinea pigs with exactly eight bacilli every day and then every three days for four months. At the postmortems lesions showing extraordinary resistance were found.

From all the evidence of experiments quoted, and from the "law of Marfan" Calmette concludes that the consumptive is one who has received since childhood *successive more or less massive reinfections*.

The contradiction between Bruyant's results with guinea pigs and

Calmette's with cattle are, to my mind, explained by the supremely important factor of dosage, to which we shall return later. Apparently, small repeated infections protect, while large ones overwhelm.

Bushnell is very emphatic that reinfection from without cannot occur after a primary infection is established. "The inference is supported by analogy with the facts of other infectious diseases; in malaria there is no reinfection with organisms of the same type, in syphilis reinfection does not occur until the disease has become cured. There is no good reason why disease caused by the virulent and highly resistant tubercle bacillus should form an exception to the law that reinfections do not take place so long as the infectious agent is present." The analogy here seems to me dubious. It is certainly reasonable to suppose that in malaria and syphilis, or in typhoid, or any other truly septicemic disease, where the infecting organism is likely to be present throughout the body in stupendous numbers, any new invasion will be insignificant. It is by no means certain that the same is true of tuberculosis, where the tendency to localization is so much more pronounced, and the general immune reactions of such uncertain effectiveness. Possibly the phenomenon of staphylococcus boils may provide a closer analogy to tuberculosis. Here the body appears to submit repeatedly to a reinfection from the surface, though it is possible that there is at times also a bacteremia without lesions of the deeper tissues, a reinfection which it is able to localize, but against which it may not succeed in developing a conclusive general immunity. Granted, however, that all reasoning from analogy is perilous.

Bushnell says further, "The subject may die of an extension of his disease, but in pulmonary tuberculosis will preserve until the close of life the first degree of immunity, that is, he will develop no distant foci due to his own bacilli, and will be immune to incursions of tubercle bacilli from without." It would seem that possibly he has overlooked the frequent ischio-rectal abscesses and laryngeal lesions which develop by no means always as terminal lesions.

Theobald Smith,<sup>7</sup> whose profound studies in tuberculosis, and whose clear vision regarding its problems, invite the greatest trust, has expressed himself very clearly on this problem of reinfection. Referring to Römer's work he says, "The resistance of the tuberculous animal to superinfection is readily broken down by slightly increased dosage, and is successful only when very minute doses come into play, as pointed out by Römer himself. Granted that Römer's inferences are correct in this, that a tuberculous focus once under way prevents the successful lodgement of the same infection in minute doses from without, we are still in the dark as to the fate of an infection of higher virulence. Römer asserts as a result of his experiments that most if not all tuberculous infection dates back to early life. This theory should be accepted with caution and reserve, although the large number of positive tuberculin tests in children give support. My own view is that a bacillus coming from without into the lungs has as good a chance, other things being equal, as one carried there from some existing internal focus. If the internal infection is massive and the fresh infection is light, the former



is more likely to produce a focus of disease than the latter. If the fresh infection is massive and the other light, the fresh infection is likely to produce disease rather than is the metastatic process."

The more we study the family history in enlightened patients, the more we realize the certainty of repeated infection in our consumptive patients. Such infections may be differently spaced, but are surely in most instances multiple, and comparable with some of the experiments—especially that with cattle—quoted in Calmette's book. The histories referred to are usually histories of family exposure and it is probably true that it is multiple—we may say cumulative infection—from without in the early years of life rather than in adult life which is important. This is indeed Bushnell's view, for in speaking of childhood infection he adds, "The especial danger of infection from tuberculous members of the family, then, lies rather in the probable large size and *frequency of the infecting doses* than in much increased probability of infection per se."

Tuberculization of the race is for the most part beneficial, as Bushnell claims, and humanity may be protected to a considerable degree by the primary infection. However, it does not as yet seem possible to be absolutely certain that reinfection from without does not occur.

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—G. B. W.

### *Treatment of Exophthalmic Goiter*

RECENT literature contains some brilliant discussions and reports of most interesting experiments on the treatment of exophthalmic goiter. A definite or unquestioned remedy for all cases has not been found or accepted but the remarkable results obtained certainly carry with it promises of relief to all.

The most promising of all experiments appear to be those of O. P. Kimball and David Marine.<sup>1</sup> The results of their experiments show that simple goiter in man may be prevented on a large scale by the simple means of administering 2 grams of sodium iodide twice yearly, dividing this into 10 doses. They found that where no enlargement of the thyroid existed previous to the first administration of this drug none developed later whereas in a similar number of children, not so treated, 15 per cent developed simple goiter. Although this work concerns only simple goiter there is at least suggestive evidence that all forms of goiter may be prevented in the same way, as the limitations of this simple preventative have not yet been determined.

There are some who believe that exophthalmic goiter is a self limiting disease and that spontaneous cures occur at the end of 5-6 years but, even if this were true in a large percentage of cases, there would be few patients willing to trust to such luck and wait until this period is past. So then, if we attempt to calculate in percentage the cures accomplished by the different methods it must be remembered that our figures are only approximately correct.

Much experimental effort has been directed towards determining, if possible, the exact etiology, with only this much of success that we can say that we have in all cases to deal with hyperfunction of the thyroid gland as one of the causes, but whether this is due to primary changes within the gland itself or to outside stimulation from its nerve supply or from any of the other glands of internal secretory type we do not know.

That a large number of complete cures follow partial removal of the thyroid gland is a well known fact and as no recurrence of symptoms follows it seems reasonable to suppose that the cause has been found and properly dealt with. That others suffering from the same disease and treated in an identical manner either without results or with recurrence of symptoms suggests equally plainly that we have here an outside causative factor which has not been reached.

Such outside causative factors have been found in the thymus gland and the cervical sympathetic ganglia from which the thyroid receives its entire nerve supply. The intimate relation of the entire group of glands of internal secretion makes it possible that even if the symptom complex is quite constant we may have the cause located in different glands of the endocrine group in the different individuals.

Capelle reported a hyperplastic thymus gland in 95 per cent of those dying from "heart failure" during or shortly after an operation on the thyroid for exophthalmic goiter. Mattie found hyperplasia of the thymus in 74 per cent of his series of exophthalmic goiter. Kocher found hyperplastic thymus in 50 per cent of his operative cases. Although the findings vary greatly in percentage there is nevertheless definite suggestion of a close relation between the disease and the thymus. Zinorzsky treated 20 cases of exophthalmic goiter with x-ray applied only to the thymus gland and found some relief of symptoms in all cases. Waters made similar experiments and of his 16 completed cases he reports 50 per cent complete cures and 43 per cent of marked improvement. Of 9 cases which received  $\frac{2}{3}$  of the entire treatment 2 were completely cured and 5 showed marked improvement. Twenty-four others received only  $\frac{1}{3}$  of the prescribed treatment and some of these showed marked improvement.

Haberer reported a case of a man who presented himself apparently in a dying condition, cyanotic, dyspneic, covered with cold sweat and without perceptible pulse in the peripheral arteries. There was marked exophthalmus, lungs were edematous, heart and liver enlarged. One lobe of the thyroid removed one year previously, arteries on the other side tied. Haberer removed a piece of tissue from behind the manubrium 3 cm. long  $1\frac{1}{2}$  cm. thick which resembled fat and microscopically did not seem to contain thymus

tissue. The patient began to improve and a complete cure followed in 3 months time.

Mannaberg applied x-ray to the ovaries in patients with exophthalmic goiter and in 8 cases of prolonged treatment he observed marked improvement. No complete cures were reported but there was marked decrease in the pulse rate, relief from diarrhea and improvement in the nervous symptoms.

Cannon succeeded in producing some of the symptoms of exophthalmic goiter in the cat by constant stimulation of the thyroid through the cervical sympathetic. Wilson found a pronounced and constant lesion of the cervical sympathetic ganglia in all cases of exophthalmic goiter examined and he states that there is much suggestive evidence that these changes are the result of direct bacterial infection within the ganglia. Leriche resected the sheath of the superior thyroid artery from its origin to the pole of the thyroid gland thus destroying the nerve supply to this lobe. The involved lobe of the thyroid gland decreased in size in a month's time so that there was no palpable tumor left, whereas the lobe on the other side remained enlarged as before.

These represent but a few of the many experiments reported in the literature, which, taken together tend to convince one that it is far from correct to suppose that the thyroid gland is or contains the sole cause of the disease.

The most logical way of treating Graves' disease would be by some method or agent with which one could attack all possible causes. Up to the present time the x-ray seems to have given the best results. The results of x-ray therapy in this disease vary in the hands of different workers and it appears that better success could have been obtained in many cases if heavier doses had been given. Nordentoft states that in his experience large doses are beneficial from the start and that small doses often irritate and many cause harm. Phaler of Philadelphia, who has done so much for Roentgen therapy, has outlined pretty thoroughly the technic to be used in this field and, with the present uniformity of apparatus, it should be possible to duplicate his results. Remer has shown that with certain distance, milliamperage, spark gap and time, and the modern broad focus Coolidge tube, results are uniform, regardless of the make of transformer or the age and discoloration of the tube used. It seems then that the results should be just as constant as the action of any drug. Holmes and Merrill in their discussion of this subject emphasize the fact that in order to obtain good results one must have a correct diagnosis and that attempts to reduce the size of a cystic or calcareous goiter would only end in disappointment and, probably, hypothyroidism. Cotenot reports excellent results from large doses with heavy infiltration in the typical cases. The nervous and heart symptoms show the first and greatest improvement, the goiter itself and the exophthalmus are the last to disappear.

McElfatric has summed up well the benefits of x-ray therapy in exophthalmic goiter as compared with other methods of treatment. There are no fatalities, no postoperative scars. It does not interfere with a patient's occupation, is painless and causes no inconvenience. If unsuccessful surgery

can still be performed and with less risk because of the favorable action of the rays of the thymus.

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—C. C. B. (P. G. W.)

### *Scurvy in Northern Russia*

COMRIE,<sup>1</sup> with the British Troops in Northern Russia in 1919, saw and was able to treat a large number of cases of scurvy which appeared among the natives and prisoners at Archangel in February. The prison dietary which was in operation for more than a year, was as follows:

Flour or biscuit	11 ounces
Rice, oatmeal, peas or beans	7½ "
Frozen or tinned meat, or salt-herring	7½ "
Bacon or pork	1¾ "
Tea	¼ "
Sugar	1 "
Salt	¾ "
Lime juice (preserved)	½ "

It will be evident that the above diet was altogether too limited, being deficient not only in proteins, but in carbohydrates and vegetables. The preserved lime juice had no effect in preventing the development of scurvy; possibly it might have been of some value in furnishing a small amount of alkali. All the meats were boiled for three hours. After men had been living on the above given diet for between four and five months they developed scurvy. The development of this disease was probably enhanced by the crowded condition of the prisons, the bad surroundings, and the darkness of the long Arctic winter. Besides, there were other diseases, such as typhus, typhoid, influenza, and pneumonia, which possibly had some influence upon the rapidity with which the scurvy developed. As a rule, the first sign was a purpuric rash on the legs. As this came out the pa-

<sup>1</sup>Edinburgh Medical Journal, April, 1920.

tient complained of mental depression, loss of energy, and physical weakness. Soon the gums were swollen and bleeding. The ankles became edematous and complaint was made of pain in various parts of the body, especially in the head, chest, or legs. About eighty per cent of the cases showed the gums tender, swollen, often ulcerated, and frequently bleeding. In many there was an extensive pyorrhea, the teeth being loose and surrounded by pockets of pus. About eighty-six per cent showed well marked hemorrhages and these were at points on the body most frequently subjected to pressure, like the trochanter, elbow, shoulder, and in localities where blows had been received. In about fifty per cent of the cases hemorrhage was found deep in the muscles, usually in the legs, accompanied by tenderness on pressure, with pain. They caused marked limitation in the movements of the limbs. It was somewhat difficult to distinguish hemorrhages into the joints from those about the joints. The joints were frequently swollen, tender, and constantly kept, so far as possible, immobile. These joint affections were in evidence not only among the prisoners, but among the natives in civil hospitals. During recovery the hemorrhages into the muscles were absorbed with striking rapidity, large black and hardened areas of from four to five inches in diameter disappearing completely in two or three weeks. Hemorrhages into internal organs also occurred and were in evidence in those cases which came to autopsy. In two cases there was hemorrhage from the bowel and in two others from the urinary passages during life. In all cases the heart beat was feeble and in many functional murmurs were easily detected. At autopsy the cardiac muscle showed a high degree of brown atrophy with great reduction in the size of the ventricles. In some cases sudden death resulted from effusions in the pleural cavities. Anemia marked the more advanced cases. It had the characteristics of a secondary anemia with lymphocytosis. It was rather surprising to find that the coagulation time of the blood was not appreciably altered and that there was no tendency to hemorrhage when superficial wounds were inflicted. There was a slight diminution in the alkalinity of the blood. The urine showed but little or no abnormality, albuminuria being observed only five times. In four cases out of fifty the urine was alkaline.

There was no difficulty in making a diagnosis of the advanced cases, but the disease was not recognized in its incipency. In early cases there might be nothing more than a rash on the legs with more or less edema, accompanied usually by mental depression. Cases at this stage were diagnosed under widely different heads.

Under the weather conditions existing in northern Russia it was found that scurvy was easily prevented by issuing a ration of germinated peas or beans. For germination the dry peas or beans are steeped in water for from one to two days until the embryonic root begins to sprout. They are then spread on one damp cloth and covered by another and kept in this state at proper temperature until the sprouts become about half an inch long. These germinated peas are lightly boiled with milk or water not exceeding half an hour. Beans do not germinate so easily, and therefore are less suitable for the army ration in cold countries than peas. Lemons or fresh lime juice are of value, but the preserved lime juice is without value.

For experimental purposes Comrie took forty-eight cases having the disease

with about the same degree of severity and divided them into six groups of eight each, indicated by the letters A, B, C, D, E, and F. These were segregated from one another and kept under guard. All of these men had a general diet, which consisted of bread, twelve ounces; oatmeal, two ounces; fat, one ounce; frozen meat, ten ounces, boiled for three hours; milk, two pints, boiled for one and one half hours; and ungerminated peas or beans, eight ounces, boiled for three hours. For the purpose of the test, to this common diet there was added to that of group A the juice squeezed from four ounces of fresh lemon; to group B eight ounces of germinated peas lightly boiled instead of the same quantity of ungerminated peas; group C eight ounces of germinated instead of ungerminated beans; group D ten ounces of fresh underdone meat instead of well frozen meat; group E eight ounces of tinned fruit; group F two pints of unboiled soured milk, replacing the well-boiled milk of the other groups.

After six weeks well marked improvement occurred in all six groups. Judged by the general well being and more precisely by the increase that had occurred in body weight the groups improved as follows: Group F (soured milk) showed most marked improvement, having added over fourteen per cent to the original body weight; group D (fresh meat) and group A (lemon juice) came next with an addition of between ten and eleven per cent; group B (germinated peas) seven per cent, and groups E (tinned fruit) and C (germinated beans) from six to seven per cent. The germinated beans had a tendency to cause diarrhea.

The local lesions in the mouth were washed with alum water or hydrogen peroxide, while the gums were painted with salicylate of soda dissolved in alcohol or with tincture of iodine. In order to hasten the absorption of the hemorrhages the areas were painted with tincture of iodine; then applications of hot water, combined with massage, were used. The contractures of the limbs required prolonged treatment by massage with oil inunction. Such tonics as iron, arsenic, and strychnin were found to be unnecessary. It will be seen from this that cases of scurvy recover with great rapidity with proper diet and under favorable circumstances. In the majority of instances the patients were normal practically within a month, with the exception that all the hemorrhages had not been absorbed and the contractures had not been relieved.

—I. C. I.

### *The Effects of Deficient Diets on Monkeys*

McCARRISON<sup>1</sup> at Coonoor, India, captured a lot of monkeys from the local jungles, divided them into groups and placed them on different diets, with the following results:

(1) An exclusive diet of autoclaved rice—that is to say, one deficient in suitable protein, in fat, in accessory food factors of all three classes, and excessively rich in starch. Ten monkeys were fed to the point of death on this diet.

(2) A diet of autoclaved rice and butter—that is to say, one deficient in

<sup>1</sup>British Medical Journal, February 21, 1920.

suitable protein, in accessory food factors of the B and C classes and excessively rich in starch as well as fat. Four monkeys were fed on this diet to the point of death.

(3) A diet of autoclaved food—rice, wheaten bread, milk and ground nuts—to which a small ration of fresh onion was added; that is to say, a diet deficient in accessory food factors of the A and B classes. Six monkeys were fed to the point of death on this diet.

(4) A diet of autoclaved food—rice, wheaten bread, milk and ground nuts—to which fresh onion and fresh butter were added; that is to say, a diet deficient only in accessory food factors of the B class and excessively rich in fats. Five monkeys were fed chiefly on this diet to the point of death.

(5) Nine monkeys fed on wheaten bread, plantains, milk, fresh onions and ground nuts, acted as controls.

It is worthy of note that the animals which received rice plus butter lost weight faster and died quicker than those that received rice only. One of the most interesting points brought out in this investigation is that those animals kept on a deficient diet developed dysentery. For a day or two the stools were diarrheal and then they became dysenteric, being followed with mucus streaked with blood. Finally, in many animals for a time the stools consisted solely of blood and mucus. In two cases the dysentery was not preceded by diarrhea. As a rule, the animals survived the onset of the dysentery for only a few days, from four to seven. The symptoms were those of amebic dysentery and amebae were found in the stools. In order to determine whether these monkeys were carriers of amebae, before the experiment was begun the feces of eight healthy animals were examined and a few amebae were found in only one of these. During the greater part of the experiment the control monkeys remained free from gastrointestinal disturbances of any kind. Towards the close, however, seven developed jaundice thought to be due to a too generous provision of monkey-nuts with lack of exercise. Under a more meager diet of bread and milk to which a pinch of Epsom salts was added after a few days the jaundice cleared up. In no control monkey did either diarrhea or dysentery occur. This is a matter of great importance. One would conclude from these investigations that in many instances at least, there are two factors in the causation of amebic dysentery. One is the parasite and the other is the food. The author calls attention to the fact that it is a common practice, especially in India, for people, either through the physician's advice or on their own initiative, to confine themselves to bread and butter and milk puddings when they have any form of dysentery. The author states:

"It is my experience in India that the dietetic history of European sufferers from chronic colitis, and all those suffering from chronic gastrointestinal disorder, commonly reveals the fact that their food does not contain the requisite proteins or the due proportion of starch, fats, salts, water, and vitamins. This one cannot 'digest' vegetables, fruit, or meat, or 'never touches them in India;' that one can 'carry on only on farinaceous food.' Thus the form of diet they commonly adopt is often that most calculated to promote the very disorder from which they seek relief. These experiments show that the cardinal effects of deficient and ill-balanced dietaric in monkeys are gastrointestinal disorder, dilatation of the stomach, gastritis, and colitis. They are likely to be the same in man.

It did not surprise me, therefore, to find that a patient who consulted me recently, and who for ten years had subsisted mainly on milk puddings, had a dilated stomach, air locks in the small bowel which caused her great discomfort, delay in the passage of the intestinal contents, colitis, tenderness in the caecal region, and an inefficient pancreas with glycosuria. The results of these experiments have helped me to visualize the changes which are likely to be occurring in the gastrointestinal tract in such a chronic invalid. I desire, therefore, to emphasize the importance in practice of a study of the dietetic history in such cases, believing as I now do that bacterial agencies are often but weeds which flourish in soil made ready for them by dietetic defects, and believing also that in the fuller comprehension of the science of dietetics we shall understand more perfectly the beginning of disease and its therapy."

Postmortem examination showed practically all the lesions characteristic of acute amebic dysentery. There were found congestion and inflammation of the mucous membrane of small intestines, with frequent subserous ecchymoses. Necrotic changes in the mucous membrane of the stomach were found and ecchymoses were frequently present at its pyloric end. In one case an ulcer was found at the pylorus. There were areas of congestion, more or less marked throughout the small intestine. In the large intestine there was marked inflammatory change, sometimes extending throughout the length of the large bowel, but more marked in the lower six inches. In rare instances the colitis was limited to areas of the transverse colon. In these areas there was pronounced ballooning of the intestinal walls.

—I. C. I.

### *From the American Society for the Control of Cancer, New York City*

**F**OLLOWING is a statement made by Dr. Harvey R. Gaylord, one of the Directors of this Society and Director of the State Institute for the Study of Malignant Disease, Buffalo, New York:

"The people of the State of New York will want to receive a statement on the stewardship of the purchase of 21<sub>4</sub> grams of radium, for which \$225,000 was appropriated by the State, and announcement of which was made by Governor Smith a few days ago.

"I am very glad to take this opportunity both in the name of the Institute for the Study of Malignant Disease, the State and the American Society for the Control of Cancer which supported this purchase to say these words:

"The experiment in state ownership of a therapeutic agent, as exemplified in the purchase of this radium for social utility will have a far reaching effect. This is a development of state medicine to which no one can object and Governor Smith deserves the thanks of the State for what he did.

"Any citizen of the United States may avail himself gratuitously after October 15th of treatment with the 21<sub>4</sub> grams valued at \$225,000, recently purchased by New York State, and the first gram of which was delivered by the Radio Chemical Corporation of New York last week. Preference, however, will be given to citizens of New York State.



"The first gram is now in the vaults of the Institute at Buffalo and the appliances necessary for its use in the treatment of cancer are now in course of construction. The engagement of a competent physicist to work with this radium is also announced. The radium we are using is an American product, mined in Colorado, brought 2900 miles across the continent in the form of 125 tons of carnotite ore to the extraction plant at Orange, N. J., where it was reduced by fractional crystallization to its present state.

"The first purchase of radium by any State marks a step in the health activities of an American Commonwealth. Up to the present we have had no therapeutic agents so expensive that they could not be afforded by the average practitioner. In the case of radium that condition arises. The unit for efficient use costs not less than \$12,000 and represents 100 mgs. A gram is worth \$120,000. The greater the quantity in an installation the more efficient it is, and the less it costs per treatment. New York State has met this condition by purchasing an amount available for all its citizens.

The value of radium has already arrived at a stage where states, and if necessary the government, should make radium available for cancer treatment gratuitously and beyond the realm of financial limitations. The advent of radium as a therapeutic measure is the most important forward step in the treatment of cancer.

"It is not surprising that when radium first made its appearance over-optimistic claims for its use and hope of its utility should have occurred. But that time is now past. Radium has been made available in smaller and larger amounts to all of the important centers of cancer research in this country, with the result that not alone has new knowledge of this agent been greatly advanced but the technic of its use, as well as its limitations, have been more definitely defined. The last six years have marked steady progress in its application, and means of more scientifically and more efficaciously employing it have been developed.

"The State Institute, as a result of carefully controlled scientific experiment in its hospital, felt that the time had come when the State of New York should logically provide an adequate amount of radium for the Institute on the basis that its value is so definitely demonstrated that it should be made available without cost to the citizens of the State and that the opportunities for research should now be extended along practical lines. The State Institute has had since 1914 an amount of radium sufficient for scientific study. Private philanthropy has given the Memorial Hospital in New York City a large amount of radium for scientific investigation and practical application for the past four years. The Cancer Research Commission of Harvard University has also had an adequate working supply. The advances made in these and other quarters has steadily strengthened the confidence in the use of this agent and all of these centers are now seeking means to increase their supply.

"The State of New York which in 1898 took the lead by founding the first modern State Cancer Research Institute in this country should properly be made the first state to appropriate the necessary funds for the purchase of a sufficient amount of radium for the use of its citizens, having available for this purpose a center of cancer knowledge and fully equipped scientific re-

search laboratories where its use can be made immediately effective, and from which scientific progress can be confidently anticipated.

"The usefulness of radium in the treatment of neoplasms is still in its infancy, but there are already certain kinds of cancer in which its use offers advantages and the results obtained are an improvement upon any means we have heretofore possessed. It must, however, be remembered that our main reliance in the treatment of cancer is surgery, but radium in combination with surgery, frequently greatly improves the prospective cure.

"The scientific development of the last two years in the use of radium, largely through the work of Professor William Duane of Harvard University, made available a means of using radium which has immensely strengthened its usefulness. This method is the use of the emanation of radium in place of the application of radium itself. This method is only available when you have at least one gram.

"Cancer today is one of the most important diseases in the United States. It increases 25 per cent every ten years. In the United States 90,000 deaths occur yearly from it, being of equal importance to tuberculosis. In New York State about 8000 deaths occur yearly.

"The purchase of the radium has other significance than merely its use for the treatment of cancer. It gives an opportunity for research and its use under scientific conditions is sure to increase our knowledge of cancer. While surgery still remains our main reliance in the fight against cancer we can only hope greatly to improve the results of surgery by bringing the patient to surgical treatment at the earliest possible moment. This can only be accomplished by the diffusion of knowledge among the laity of the first beginnings of cancer. It is with such work as this, that the Society for the Control of Cancer has particularly charged itself. It is felt by the Society that the advent of an alternative will overcome the reluctance of many cases to present themselves to their physicians. The Society represents 900 physicians and laymen and looks with great interest at the purchase and congratulates New York upon the step it has taken.

"The purchase of this radium by an American Commonwealth from an American Company which has mined its ore in the State of Colorado, will bring still further to the fore the preeminence of America in the treatment of cancer. Buffalo will become a radium center. While Europe, through Madam Curie, first made the precious element known to the world, the United States has developed both the ore, its extraction and its use as a therapeutic agent. It is today in the forefront of treatment of cancer. This purchase may have a tremendous effect upon further progress in this direction."

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## ORIGINAL ARTICLES

### THE ETIOLOGY OF ACUTE INFLAMMATIONS OF THE NOSE, PHARYNX AND TONSILS\*

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#### I. INTRODUCTORY

THAT a state of confusion exists as to the etiology of the acute inflammations of the upper respiratory tract is evident upon casual excursion into the literature. A more patient search, however, reveals certain material, notably in the form of recent laboratory work, from which definite conclusions can be drawn, and in whose light the discussions of the "common cold" in the current texts is clearly seen to be obsolete. The present paper is concerned with the etiology of the acute inflammations of the nose, pharynx, and fauces, particularly with reference to recent laboratory inquiries. The question of the excitation of sporadic infections by exposure of the body surface to cold is dwelt upon in considerable detail—much more at length than its relative importance deserves, indeed—simply because the newer data here presented is the contribution of the present authors.

Since the early days of bacteriology, attempt has been made by the several proponents and opponents of the infectious theory to refer the "common cold" on the one hand to the action of a specific microorganism, and on the other to

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The original experiments described in this paper were chiefly performed at Washington University, St. Louis, by the above authors. The paper itself is a revision of a dissertation submitted by Stuart Mudd and awarded for 1920 the Boylston Medical Prize of Harvard University, which is open to public competition. By an order adopted in 1826, the Secretary of the Boylston Medical Committee was directed to publish annually the following votes:

1. That the Board does not consider itself as approving the doctrines in any of the dissertations to which premiums may be adjudged.

2. That, in case of publication of a successful dissertation, the author be considered as bound to print the above vote in connection therewith.

various environmental and constitutional causes, such as exposure to changes of temperature, the "lithemic diathesis," and what not. Although perhaps laudable as philosophic ideals, such efforts to explain the many phenomena involved by a single cause are less deserving scientifically, and have met with just failure. The "common cold" is, as a matter of fact, in most instances the result of a local infection, but there are many types of "cold" and many infectious agents responsible for them; and the effect of various constitutional and environmental factors in determining infection is often of great importance. Furthermore there are many acute inflammations of the upper respiratory tract not primarily due to the local action of microorganisms, but rather the local expression of chemical or mechanical irritation, of thermal trauma, of nervous reflexes, of drug intoxications, of constitutional disease, or of anaphylaxis.

## II. BACTERIOLOGY OF THE COMMON COLD

The studies of a number of investigators have shown that, although the entrance of the nose is swarming with bacteria, the flora of the nasal cavities proper in health is kept exceedingly sparse by the action of the ciliated epithelium, the trickling of the lacrimal and mucous secretions, the inhibitory action of the mucus, and by phagocytosis. (Thomson, 1913, p. 7.) The folds of the pharyngeal mucosa and especially the crypts of the tonsils are, on the other hand, known to maintain even in health an abundant flora, including often potentially pathogenic organisms (e.g., see Davis, 1920). Bacteriologic studies of the nose and throat in the course of acute "colds" have usually shown the presence of microorganisms in unwontedly large numbers, often with one form so predominating as to suggest for it an etiologic relationship with the cold. In a number of instances immunologic and inoculation data have supported the bacteriologic evidence and would seem to warrant assumption of a causal relationship between the bacteria and the colds in question.

In 1873 Hüter described a micrococcus as a cause of coryza (cited by Benham, 1906).

Hajek (1888) described a large diplococcus, the *Diplococcus coryza*, in the early stages of acute colds. He advances no other evidence in support of his bacteriologic findings, however. Certainly in the light of recent experience with influenza, the mere presence of bacteria in large numbers during an inflammation cannot be regarded as valid ground for considering them its cause. (For a discussion of bacteriologic methods in estimating the relationship of microorganisms to respiratory disease, see Allen, 1913.)

Paulsen (1890), examining twenty-four cases of cold of diverse clinical types, some with history of exposure to other persons with colds, and others following exposure to chilling, found various cocci and bacilli as the predominating organisms.

Cautley (1894-95) found an aerobic diphtheroid bacillus, oval, with a tendency to polar segregation of the protoplasm, in seven of eight cases of acute cold of various types. The infection started in trachea, pharynx or nose. Cautley, believing the organism responsible for coryza, called it *B. coryzae segmentosus*. Gordon (quoted by Benham, 1906) found Cautley's bacillus in all of seven cases of cold. Gordon injected two guinea pigs with the organism

and noticed they were sick a few days, but recovered. R. Prosser White (quoted by Benham, 1906) found Cautley's bacillus in seventeen of twenty-one cases. Inoculation experiments upon the noses and genital tracts of guinea pigs, rabbits and monkeys were negative.

Benham (1906) found a diphtheroid organism he regards as identical with Cautley's bacillus in twenty of twenty-one cases of cold. *Micrococcus catarrhalis* was also present in many cases. He concludes "in view of the fact that nasal discharge was not a prominent feature of my series of cases, it seems likely that the diphtheroid organisms are rather a cause of a painful sore throat with headache, malaise and muscular pains, irritable cough and scanty viscid expectoration. Whether they cause coryza—a cold in the head—is at least open to question, especially in view of the presence of *M. catarrhalis* in nearly half my cases." Benham therefore gives Cautley's *B. coryzae segmentosus* the less committal name of *Bacillus septus*.

*Micrococcus catarrhalis* was found by R. Pfeiffer (quoted by Neisser in Kolle and Wassermann's "Pathogenen Mikroorganismen," 1913) in enormous numbers in the sputum of an epidemic of mildly febrile cases of bronchitis. Gohn and H. Pfeiffer (1902) found this diplococcus in eighty-one of one hundred forty cases of respiratory tract infection. They regarded it as a saprophyte which can under appropriate circumstances give rise to acute or subacute infection. Bezançon and de Jong (1905) found this organism very frequently in an influenza-like epidemic in Paris. Along with *M. catarrhalis* they found *Micrococcus paratetragenus*, pneumococcus, Friedländer's bacillus, staphylococcus, streptococcus and diphtheroids. Dunn and Gordon (1905) believed the *Micrococcus catarrhalis* to be the chief organism in a severe epidemic in Hertfordshire with clinical manifestations in different cases simulating cerebrospinal fever, influenza and scarlatina. Allen (1906) made claim to have isolated *M. catarrhalis* with ease from each case examined in a severe local epidemic of colds in England.

Neumann, (1902) as a result of a considerably more extensive and thorough bacteriologic study than the foregoing, concluded that virulent *diphtheria bacilli* and the *pneumococcus* at least, perhaps other organisms, could produce the ordinary cold.

He summarises as follows the results of his study of the flora of the noses of 111 normal persons and of 95 suffering from nasal affections of various sorts:

"The total number of bacterial species found was 19. Nevertheless in most cases there are relatively few different species found present together. Most frequently are found diphtheroid bacilli and white micrococci. Less frequently orange, gray and yellow micrococci, pneumococci, streptococci, Friedländer's bacilli, diphtheria bacilli, isolated colon bacilli, yeast, molds, mixed bacilli, sarcinae and still a few other organisms.

"*Micrococcus pyogenes albus* is present in 86 to 90 per cent, diphtheroids in 98 per cent of the cases, so that one can justly assert that the latter occur in every sound and pathological nose. The more delicate form (*B. xerosis*) is much more frequent than the more luxuriantly growing form (Hoffman's bacillus.)

"In colds the pathogenic organisms, pneumococcus, Friedländer's bacillus, streptococcus pyogenes and diphtheria bacillus are more prominent than in normal noses.

"The diphtheroid bacillus is not virulent. Seventy-eight strains cultivated from different noses in no case killed guinea pigs. In a few cases only weak infiltrations appeared at

the site of injection. The organism cannot be brought into relationship with the origination of the cold and is only to be considered as a harmless saprophyte.

"Certainly it is demonstrated that virulent diphtheroid bacilli and Fränkel's pneumococci can cause the clinical picture of the common cold. Whether and in what way other pathogenic germs are concerned in it, is still to be answered.

"A specific cause of the cold has not been found in the investigations."

Claims for the *Bacillus of Friedländer* as a cause of "cold" are advanced, with immunologic support, by Allen (1906). Allen asserts that in each of two epidemics of a severely infective character in every case examined within the first 24 hours, and in some later, the bacillus was found. "It was of a very virulent stamp, being pathogenic not only for mice and guinea pigs but even for rabbits; it also clotted milk and fermented broth with ease."

Claims for an etiologic relationship are based upon the following statements, which, if true, would seem to establish the point:

"The appearance of the bacilli in the nasal passages of the people affected synchronized with the onset of the attack.

"The organism and colds disappeared together.

"The opsonic index of the patient's blood, which was particularly studied to the bacillus of Friedländer was affected by a cold precisely in the way that would be expected in the case of an infection by that organism, that is it rose steadily to a maximum, remained there for some time, then steadily fell to about unity during a period of perfect freedom from cold. Second and third attacks had precisely similar results.

"The appearance in the house of a person whose nasal passages were known to be infected by the bacillus of Friedländer sufficed to start an epidemic of colds on several occasions; and from the noses of such as were examined, the bacillus of Friedländer was also isolated."

Work somewhat more convincing than any of the foregoing has been reported by Tunncliffe (1913, 1915) and Howell (1915). The organism is an anaerobic curved bacillus. *B. rhinitis*, Tunncliffe.

Miss Tunncliffe, working in Chicago, found her bacillus present in 6 per cent of some 86 normal noses examined, in 98 per cent of 82 cases of acute coryza, and in 90 per cent of twenty odd cases of chronic rhinitis with mucoid discharge.

A slight rhinitis was produced three times in human subjects by swabbing a nose free from *B. rhinitis* with a pure culture. The infection began from 6 to 8 hours after the inoculation and lasted about 48 hours. The organisms were present in fairly large numbers in the nose and pharynx (in the cases with pharyngitis) 18 hours after the inoculation, and persisted for three days in two of the cases. Cultures were made twice, and the organisms isolated in pure culture both times. The opsonic index was taken during two of the infections. Both times it fell below normal, rising high above normal as the infection disappeared.

Vaccination of two patients with *B. rhinitis* produced a primary depression of the opsonic index followed by a rise above normal.

Miss Howell found that, using *B. rhinitis* as antigen, fixation of complement is obtained with the sera of persons with acute rhinitis and of persons injected with the bacillus after it is killed by heat. The fixation is most marked a few days after the onset of the infection and lasts only a short time. Sera

of normal persons and of patients with various infectious diseases do not give complement fixation with the *Bacillus rhinitis*.

It is difficult to escape the conclusion that *B. rhinitis* was causally related to these cases of acute coryza about Chicago.

That *streptococci* may be responsible for infections of the nose, and more especially the pharynx and tonsils, has been shown by many authors (vide Lingsheim 1912, and Barnes, 1914, p. 67).

Mathers (1917) made a careful bacteriologic study, using aerobic, anaerobic and filtration methods, of an epidemic of acute respiratory infection in Chicago, which resembled the influenza of 1889-92. He concluded that a virulent hemolytic streptococcus was the probable cause.

Floyd (1920), as a result of bacteriologic study and vaccine therapy of the winter colds occurring in and around Boston, concluded that in acute rhinitis the organism commonly found belongs to the staphylococcus group, and that somewhat less frequently the hemolytic streptococcus has appeared. In acute pharyngitis "almost invariably the initial infection is produced by the member of the streptococcus group."

*Pneumococcus* has also frequently been recognized as concerned with infections of the upper respiratory tract. (v. Abel, 1892, Neufeld u. Händel, 1912; Floyd, 1920.)

Discussion of *B. influenza* has purposely been left out of this paper. Under this caption, however, may be quoted the conclusions of R. W. Allen (1913) based upon some ten years of bacteriologic study of the respiratory diseases, in England.

"To summarize," he writes, "it would appear that any of the seven organisms, *B. influenza*, *pneumococcus*, *streptococcus*, *M. catarrhalis*, *M. paratetragnus*, *B. septus*, and bacillus of Friedländer, alone or in varying combinations, may be responsible for a catarrhal condition of the upper respiratory passages. In perhaps 40 per cent of cases one organism so predominates as to justify the conclusion that it is the cause of the attack; more often two or more organisms are associated together, the *B. influenza* with the *pneumococcus* or *M. paratetragnus*, the *B. septus* with the *M. catarrhalis* or *M. paratetragnus*, so that it becomes very difficult to decide which organisms stand in a directly causal relationship to the attack. My own belief is that mixed infections from the beginning are fairly common.

"In uncomplicated purulent nasal catarrh the streptococcus is the most frequent cause, next to it the *Staphylococcus aureus*. When sinus complications coexist, the *B. influenza* and *pneumococcus* are by far the most frequent bacteria concerned."

Blake and Cecil (1920) have also recently reported successful infection of monkeys with *B. influenza* introduced both into the nose and intratracheally.

Although any attempt at adequate discussion of the etiology of epidemic influenza is outside the scope of the present paper, its virus must be included among those capable of giving rise to acute inflammation of the upper respiratory tract. We would express our own opinion, too, that the attributes of a specific, labile, and elusive virus, to which so many converging lines of evidence have pointed, are extraordinarily well filled by the *filterable virus* which has been the object of the past two years of careful study by Olitsky and Gates at the Rockefeller Institute (Olitsky and Gates, 1920). The results of these studies will be further presented by these authors in the February

and March, 1921, numbers of the *Journal of Experimental Medicine*. For a bibliography and brief review of "The Epidemiology and Etiology of Influenza" reference is made to A. J. McLaughlin (1920).

A *filterable virus* seems without question to be the causative agent in the coryza of one fairly well defined type. Kruse (1914) diluted the secretion from the nose of an assistant with coryza, passed it through a small Berkefeld filter, and dropped a few drops of the filtrate on the nasal mucosa of each of twelve men. Four of them developed colds in from one to four days. In a second experiment 42 per cent of thirty-six subjects so inoculated developed coryza within from one to four days. Kruse could find no living organisms in his filtrates by bacteriologic methods.

Foster (1916) in repeating and extending this work, passed through a small Berkefeld N filter, with aseptic precautions, the nasal secretion of persons suffering from acute colds. This filtrate he proved to be free from ordinary bacteria both by aerobic and anaerobic methods. From three to six drops of the filtrate were placed in each nostril of each of ten soldiers. Nine of the ten men developed the usual symptoms of acute coryza in from eight to thirty hours. Inoculated into tubes of tissue broth or tissue ascitic fluid under petrolatum, after the method of Noguchi and Flexner with rabies and poliomyelitis virus, this filtrate produced a growth which could be subcultured apparently indefinitely. Specimens of these subcultures were again diluted, passed through a Berkefeld filter, and a few drops allowed to run into the noses of eleven healthy soldiers, every one of whom developed, after an incubation period of eight to forty-eight hours, acute colds. Cultures from these individuals' nasal secretions gave the same appearance as the original cultures. They contained an abundant growth of a pleomorphic virus varying from minute globoid bodies to coccoid forms larger than staphylococci, the latter apparently in some instances with small globoid buds. (Foster 1917.)

The clinical picture described by Foster both for his original patients and for the subjects of the inoculation experiments is worth giving by a typical case. The patient "complained of lassitude, chilly sensations, sneezing, unilateral nasal stuffiness, dull frontal headache with a feeling of oppression over the eyes, impairment of smell and moderate aching pain in the extremities. There was the usual lacrimation, a copious, thin, mucoid nasal discharge which excoriated the upper lip and the alae of the nose, and a very red, moist, swollen and boggy mucosa. The temperature was normal."

The contention of Professor M. J. Rosenau, in whose laboratory Foster's work was done, that this type of acute coryza deserves to be considered a clinical entity, would appear to be well founded. It is extremely interesting that a symptom-complex has been separated on purely clinical grounds from the remaining congeries of nose and throat affections. Coolidge (1918, p. 92) writes: "But there is one form of 'symptom-complex' so common and constant that it might well be classed as a distinct and definite disease, and it is to this disease that the word 'cold' is most frequently applied." His description of it closely corresponds to Foster's.

A survey of the literature then would seem to warrant the following conclusions:



1. A common and fairly well defined clinical entity, an acute coryza, exists, probably with the filterable virus of Kruse and Foster as its causative agent. This affection is readily communicable and probably does not depend to any great extent upon the action of exciting factors in depressing the resistance of the subject. 2. A heterogenous group of pure and mixed infections of the nose, pharynx and tonsils exists with various clinical pictures—some closely approaching that of Foster, others mere circumscribed inflammations—and with any one of a considerable number of bacteria capable, under appropriate circumstances, of acting as causative agents.

The microorganisms whose etiologic rôles seem to the writer to be best established are pneumococcus, streptococcus, *B. diphtheriae*, *B. rhinitis*, Friedländer's bacillus, and *B. influenzae*. Strong bacteriologic evidence, unsupported, however, so far as the writer knows, by immunologic or experimental data, has been advanced for *M. catarrhalis*, *B. septus*, *M. paratetragenus*, and *S. aureus*. The possibility that still other organisms may be primarily or secondarily involved is, of course, not excluded. Wide variations in virulence exist, both between different organisms and probably from time to time in the same bacterial strain (see Allen, 1913); some organisms are doubtless capable of causing infections in epidemic proportions, nearly or quite independent of accessory exciting factors; others may exist as harmless saprophytes upon the mucous membranes, causing infection only when the resistance of their host, local or general, is lowered by some exciting factor.

### III. EXCESSIVE CHILLING AS AN EXCITANT OF INFECTION

It is not the purpose of the present dissertation to attempt a sifting of the large amount of evidence, collected chiefly by ordinary and by clinical observation, which bears upon the question of the predisposing and exciting factors of upper respiratory infections. Suffice it to say that anything which lowers resistance, general or local, may disturb the equilibrium between host and parasite in the direction of exciting infection. The present section of this dissertation will concern itself with one particular factor only, namely, exposure to cold.

In the consideration of cold as an excitant of mucous membrane infection the issue has been somewhat confused by certain other effects of chilling. One of the immediate results of exposure, for instance, may be a transient rhinorrhea having no necessary relation to "catching cold." Again a very slight draft of cool air may, in the stage of onset of a cold, be accompanied by a feeling of chilliness or even a rigor—this is obviously an effect and not, as it is sometimes wrongly considered, an antecedent of the infection.

But, in addition, it would seem to be a fact attested by long and general experience that excessive exposure to cold may be an actual excitant of rhinitis, pharyngitis or tonsillitis. Certainly the weight of authority is in support of this thesis. Of the laryngologic texts, the following recognize chilling as an efficient factor in exciting infection: Grayson (1902), Ballenger (1908), Coakley (1914), Kyle (1914), Wright and Smith (1914), Phillips (1919), and Tilley (1919). However, Thomson (1913) and Coolidge (1918) are skeptical. In sup-

port of the affirmative are Marchand, in Krehl u. Marchand's *Handbuch d. Allgemeinen Pathologie*, (1908), Lingelsheim in Kolle u. Wassermann's *Handbuch d. Pathogenen Mikroorganismen* (1912), Packard in Osler and McRae's *Modern Medicine* (1914), Barnes, "The Tonsils" (1914), Barker, *Monographie Medicine* (1916), MacCallum's *Pathology* (1920), and Rosenau (1920). The experiments of Miller and Noble (1916), who have shown that the liability of rabbits to respiratory infection by *B. bovis septicus* is heightened by exposure to cold after overheating or by overheating after chilling are extremely suggestive in this connection. We, too, have obtained some experimental evidence (Mudd and Grant, 1919, Grant, Mudd and Goldman, 1920) as will be shown later, which would seem to afford support to the thesis that mucous membrane infection may be excited by chilling the body surface.

What would seem to be conclusive evidence in establishing excessive exposure to cold as an efficient excitant of acute upper respiratory affections has recently come from Germany in the form of statistics for large bodies of troops during and before the war. (Schade, 1919.) The data in brief is as follows:

(a) Comparison of the incidence of disease during the mild winter of 1915-1916 and during the very severe winter of 1916-1917. The increase of disease incidence rose to almost twice the total in 1916-1917; the largest single factor in this increase was the heightened incidence of respiratory affections, which in February, 1917, rose to 7.6 times the usual summer rate.

(b) Comparison of sickness among 8,000 infantry troops of whom one part (2700 men) were subjected for three days and nights to conditions of severe cold and wet, while the rest stayed behind during the same weather in village quarters. In spite of hardening by the three previous years of trench warfare, the incidence of acute respiratory diseases, "rheumatic diseases" and acute urinary affections, chiefly acute bladder irritation with diurnal and nocturnal enuresis, was four times as great among the exposed as among the sheltered troops.

(c) Comparison of sickness among 8,000 infantrymen of whom one part (4500 men) were exposed three days and nights to extreme cold ( $-9^{\circ}$  to  $-12^{\circ}$  C. midday temperature) accompanied by a sharp, gusty wind, while the remaining 3500 men were in sheltered quarters. The exposed troops comprised 160 cases of "exposure disease"—i. e., 128 acute respiratory cases, 28 of rheumatism, 4 of severe bladder irritation, and 4 of freezing. Reckoned on a basis of equal numbers of men, the sheltered troops had only 40 cases of "exposure disease" and one of freezing.

(d) Comparison of daily incidence during three months of diseases of the respiratory tract with that of "rheumatic" affections among 8,000 troops. The curves are clearly parallel. On the same days in which the number of cases of respiratory tract catarrh increased, the cases of rheumatic pain became more numerous also.

(e) Comparison of incidence of "diseases of exposure" (see above) with that of cases of freezing. Daily curves for 8000 troops show parallelism.

The sanitary reports of the Prussian war ministry, comprising all detected cases of diseases among all the men of Prussia, Saxony and Württemberg in com-

pulsory military service (more than a half million annually) have been analyzed and plotted for monthly intervals over twelve years. Of upper respiratory diseases, excluding tonsillitis, 428,714 cases are included, of "acute muscular rheumatism" 72,179 cases, and of "frost-bite" 12,898 cases. Always the upper respiratory diseases show in January and February their steep winter rise; year by year the incidence of "muscular rheumatism" and of frost-bite follow in astonishing parallelism.

The curves of incidence by month for the whole twelve years combined are also given. The curve for frost-bite rises from the base line in October steadily to a crest in February and returns to the base line in May. It is nearly symmetrical and of the form of the familiar frequency polygon. The curve for tonsillitis (271,852 cases) rises closely parallel to the frost-bite curve from December to its peak, almost identically placed in February, and descends again almost parallel until April. The tonsillitis curve is distinguished, however, by a small secondary peak in November and by its slope becoming less steep during the spring and summer so that the base line is not approached before the October rise begins. The curve for upper respiratory diseases exclusive of tonsillitis runs nearly parallel to that of tonsillitis, including the secondary mode in November, but reaches its summit in January.

It is difficult to see how one could study the curves and yet escape the conclusion of the author that "chilling by the weather and the occurrence of respiratory tract catarrh stand certainly in the closest correlation."

Such close parallelism, as Schade also gives curves to show, is not to be found between the incidence of diseases of exposure and air temperature; it is apparently with the cooling power of the air, as determined by temperature, humidity and by movement, and expressed in the frequency of frost-bites, that the parallelism exists.

Incidentally, to digress for a moment, the efficacy of the common respiratory affections in lowering resistance and preparing the way for the specific infections of mumps, scarlet fever, and measles is shown in other curves in this most illuminating paper.

It is probable that in the general population, where the physical condition does not average so high as among the young men studied by Schade, and where exposure is not so severe, the element of contagion would play a much larger and weather a much smaller part in determining incidence of respiratory affections. However, even here, excluding the great epidemics, influenza and pneumonia begin with the frost, become severe when the temperature averages below freezing, reach a maximum when the temperature is lowest and then decline. (Jour. Am. Med. Assn., editorial, 1920, lxxv, 1500.)

As to the mechanism of excitation of infection by chilling the skin, current opinion has gone curiously astray. Since the classic studies of Pasteur with anthrax and fowls with wet feet many authors have shown that animals whose blood temperatures have been lowered may show decreased resistance to bacterial infection (Marchand 1908). Trommsdorf (1906) further believed he showed in such animals a decreased motility and phagocytic activity of the leucocytes and a diminished capacity for regeneration of alexine and for elaboration of specific antibodies. (For literature on this subject, see Foord, 1918.) But, as

Marchand himself says, and as our experiments would indicate (see below), no such considerable lowering of body temperature occurs in the great majority of instances of exposure responsible for excitation of the common upper respiratory infections. Conditions in the existing animal experiments are not properly comparable to the conditions of "catching cold" in man; we must look elsewhere for an explanation.

The theory commonly advanced has been that cutaneous chilling, driving the blood inward, produces, by mechanical or reflex means, or both, congestion of the internal organs. Indeed such congestion has been demonstrated in animal experiments by a number of authors: Lassar (1880) sectioned the lungs, livers and hearts of animals after immersion in ice water, and found their vessels greatly dilated. Schüller (1881) has shown congestion of the arteries and veins of the pia mater in animals chilled by application of cold compresses. Rossbach (1901), Kisskalt (1901) and others have made similar observations upon the epithelium of the exposed trachea. Winternitz (1881) has demonstrated an increase in the volume of the arm of a human subject immersed in a cold Sitz-bath, a decrease in the arm's volume when the bath-water was warm.

Many authors have assumed that such findings apply equally well to the human nose and throat. The common observation that chilling may in a few minutes be followed by a feeling of stuffiness in the nose, has seemed to lend plausibility to such an assumption. That it is nevertheless absolutely at variance with what actually occurs in the nasal cavity, nasopharynx, oropharynx, tonsils and palate is shown by the experiments to be described below.

#### EXPERIMENTAL STUDIES OF VASOMOTOR REACTIONS OF HUMAN SUBJECTS TO CHILLING OF THE BODY SURFACE

Inquiry as to how the human upper respiratory mucous membranes are affected by chilling of the body surface has been undertaken by the present authors by methods of their own devising. (Mudd and Grant, 1919; Grant, Mudd and Goldman, 1920.) The mucous membranes of the palate, faucial tonsils, oropharynx and nasopharynx and of the nasal cavity have been studied, primarily with regard to the vasomotor changes reflexly effected in them by chilling; and in the more recent work the changes in the bacterial flora occurring during the chilling experiments have also been considered.

Methods. The experiments were performed upon human subject, for the most part third and fourth year medical students and recent graduates.

Of the several criteria of vascular condition, *heat* seemed most readily susceptible of quantitative study. Estimation by inspection of the *redness* of the mucosæ was used as a check.

To follow superficial temperature changes in the sites under consideration by the direct application of thermometers was quite impracticable. Thermogalvanometry was of necessity employed therefore.

Apparatus used: Two similar three-element thermopiles were made up of German silver wire, No. 30, and of copper wire, No. 25. In making each thermopile, three lengths of the German silver wire about a yard long were soldered alternately to three pieces of copper wire of the same length. The wires were then folded together to make a single bundle of six strands with three German

silver-copper junctions at one end for application to the surface of unknown temperature, and two junctions and a loose end of copper and another of German silver at the other end of the bundle, which was to be kept at a known temperature. To each of the loose ends was soldered a copper wire leading to a rocking key. The known-temperature end of the thermopile was packed in cotton with a sensitive thermometer in a test tube suspended in the room by a clamp about its neck, or packed in a thermos bottle containing ice water.

A second three-element thermopile was similarly arranged and connected to the rocking key. From this key copper wires were led to a D'Arsonval galvanometer. Thus, by pushing down, successively, the two ends of the rocking key, each of the thermopiles could be brought successively and separately into circuit with the galvanometer.

The unknown-temperature end of the mucous membrane thermopile, when applied, was continuously bathed in mucus, containing electrolytes. Its terminals had therefore to be insulated from each other; this was accomplished by dipping them repeatedly into an alcoholic solution of shellac. The skin thermopile was similarly protected against short circuiting by sweat. The adequacy of the insulation was proved by calibrating the thermopile both in salt solution and in distilled water.

The sensitivity of the apparatus above described was such that one millimeter deflection on the galvanometer scale indicated a temperature difference of about one-tenth degree centigrade between the two ends of the thermopile in the circuit.

For calibration, the unknown-temperature terminals of the thermopile were bound with elastic about the bulb of a sensitive thermometer and this was immersed in a suspended test tube of distilled water or salt solution.

This test tube was again suspended in a beaker of water containing a stirrer. The temperature of the outer beaker was slightly raised at intervals, the water in beaker and test tube stirred until a constant temperature had been reached, and the thermometer and galvanometer readings then taken. The calibration curves constructed from the data thus obtained were found to deviate appreciably, though very slightly, from straight lines. Temperatures were therefore taken from the curves directly.

Application of the thermopile terminals: The phase of the thermogalvanometric study of the skin and mucous surfaces which presented a new problem was that of applying the unknown temperature ends of the thermopiles. They had to be so fixed upon the site to be studied that they would remain in unchanged position, under constant and light pressure, and in such a way as not to interfere with the rise and fall of temperature in the surfaces under them in response to changing vasomotor conditions. The first two requirements were met by fastening the thermopile wires upon stiff carriers which we may designate as "applicators," and then, in the early work, fastening the latter to the appropriate surfaces by strips of adhesive plaster. Various applicators of carved wood and of wood padded with cotton covered with adhesive plaster were tried and rejected because they interfered with the normal loss of heat from the surfaces under them. Applicators satisfactory in all respects were finally made

from galvanized iron wire. A medium size was used, such that it could be twisted and hammered into the required shapes, yet would bear considerable pressure without deformation. No. 13 wire proved most satisfactory.

The several applicators used in the experiments to be considered below are shown in Figs. 1 and 2. Each consists of a body along which the thermopile

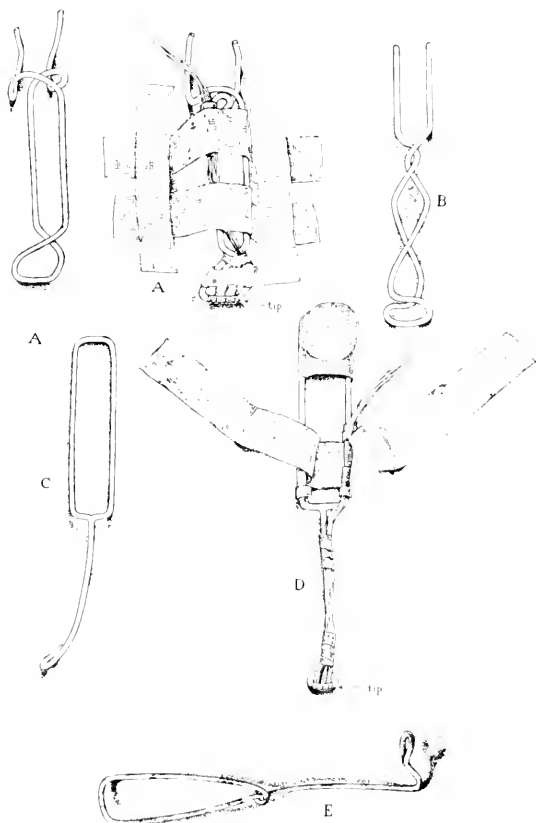


FIG. 1. Applicators. *A*, for skin; *A'*, same, applied; *B*, for soft palate; *C*, for faucial tonsils; *D*, for oropharynx, ready for application; *E*, for nasopharynx.

wires are strung and supports placed, and a tip just large enough to allow the three terminals of the thermopile to be twisted around it. The tip is so shaped as to conform to the contour of the surface against which it is to hold the thermopile terminals. The terminals, although insulated with shellac as described above, are separated from the metal tip by a single strip of adhesive

plaster as a further safeguard against short-circuiting. (See Fig. 1, *A'* and *D* and Fig. 2, *F*.)

The skin applicators (Fig. 1, *A* and *A'*) are so shaped as to form a bridge, resting stably on a support at either end, one of which is the tip, bearing the terminals. Across this raised bridge are placed the adhesive straps which hold the device in position on the skin. The tip rested usually either in the subject's supraclavicular fossa or on the forehead.

The mucous-membrane applicators (Fig. 1, *B*, *C*, *D* and *E*) were slung in the subject's open mouth by a long adhesive strip (Fig. 1, *D*), which supported the body of the applicator, then passed up just in front of the corners of the mouth and was fastened on the subject's two cheeks. The tip of the applicator held the thermopile terminals against the particular site on the palate, tonsil or pharynx whose temperature changes were to be studied. The other end of the applicator projected out of the subject's mouth and bore a small weight. Thus

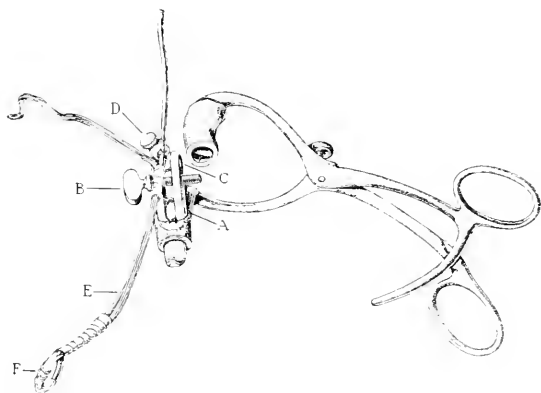


Fig. 2.—Tonsillar applicator and holder. *A* and *C*, ball and socket joints made by metal spheroids and blades of head mirror; *B*, set screw for tightening joints at *A* and *C*; *D*, set screw for fastening applicator in holder; *E*, applicator; *F*, tip bearing thermopile terminals.

the whole device when in position constituted a lever whose fulcrum was the supporting adhesive strip, whose short arm was weighted and the tip of whose long arm held the thermopile end against the mucous surface.

In the experiments upon the faucial tonsils this method was much improved upon. Into one arm of a Doyen mouth gag (Fig. 2) a small brass sphere, *A*, was screwed. A second brass sphere bearing a groove closed by a set screw, *D*, was made. The two spheres were connected by means of the blades of an ordinary head mirror, *C*. The blades could be clamped stably upon the spheres by means of a second set screw, *B*. The applicator, *E*, so shaped as to have its tip fit against the tonsil, was held in place in its groove by the set screw, *D*. By varying the shape and position of the applicator and arranging properly the two joints at *A* and *C* any desired application could be made. The subject's teeth were protected from the metal gag by rubber as shown in the drawing.

The applicator-holder for the nasal cavity experiments consisted of a plate

of strong but slightly flexible fiber-board, about 1.5 mm. in thickness, so shaped that it could be firmly held between the subject's teeth, and cross-hatched with a fine saw to prevent slipping, into which was screwed a metal sphere like that of Fig. 2, *A*, connected through metal blades to a second spheroid with groove and set screw as in Fig. 2. The arrangement of the applicator and thermopile wires was like that of Fig. 2 except that the applicator was straight and its terminal loop for the thermopile tips was very small. The subject held his lips tightly closed over the fiber-board plate.

Arrangement of the subject: The experiments were performed in a constant-temperature room, kept, on the average, between eighteen and nineteen degrees centigrade. The subject entered the room undressed save for shoes and socks, but warmly wrapped in loose garments. Throughout an experiment he sat in unchanging position, tongue held flat on the floor of his mouth, an applicator in position. The sites on which the applicators were placed were of course never wrapped and were protected from direct chilling. The experimenter removed and reapplied the wraps without disturbing the subject. Chilling was effected by (a) removing the wraps, or (b) unwrapping and applying cold wet towels to the subject's back or (c) unwrapping and turning an electric fan upon the subject's back. The third method was found to be by far the most efficacious.

In the earlier experiments very considerable difficulty was experienced because of the necessity for swallowing the saliva and mucus secreted in excessive amounts under these circumstances. This difficulty was subsequently minimized by continuously evacuating the liquids in the mouth through a glass tube—the ordinary saliva-ejector tube of the dentists—connected through rubber tubing with a suction pump on a water faucet.

Factors determining superficial temperature changes: The factors determining the temperature changes in the skin and mucous surfaces during the course of an experiment may now readily be understood. The skin is a surface constantly being heated from below by the circulating blood in the cutaneous vessels (and to a less extent by direct conduction of heat from the deeper tissues), and constantly losing heat by evaporation of moisture, and by radiation, conduction and convection to the cold air of the room to which it is exposed. Since the temperature of the room (and of the deeper body tissues) remains virtually constant throughout the experiment, the skin temperature depends primarily upon two variable factors, viz., (a) the blood temperature and (b) the amount of blood per unit of time which circulates through the cutaneous vessels. Of these the latter is incomparably the more important. For although a rise or fall in blood temperature would of course tend to effect a corresponding change in skin temperature, yet such blood-temperature changes under the conditions of our experiments were found to take place only to a relatively slight degree. It is a fact of much greater moment, that, as more blood circulates through the skin, the superficial temperature must rise, that as the cutaneous vessels are constricted the superficial temperature must fall.

For theoretical completeness, alterations in rate of evaporation of sweat must also be considered. However, with the sudden changes from warmth to chilling and vice versa



used in our experiments, sweat evaporation changes must have played a small part; and whatever part they did play must have been simply to make less striking the primarily important effects of vasoconstriction and vasodilation. For example, chilling the body surface was found experimentally to cause a sharp fall in superficial temperature due to reflex cutaneous vasoconstriction. But this same chilling would decrease the production of sweat and the rate of sweat evaporation and hence tend to prevent the fall in temperature incident to vasoconstriction; and similarly for warming the body, *mutatis mutandis*.

Also in the mucous membranes the factor of prime importance in effecting temperature changes is that of vasomotor tone. Blood temperature, because of its relative constancy, is of minor interest. Room temperature, rate of evaporation of liquids and conduction of heat to the surface from deeper tissues are all virtually constant throughout the experiment, and so require no consideration. A third factor of consequence operating here must, however, be considered—namely, changes in rate and volume of respiration. We may assume the mucous membrane, warmed by the circulating blood, to be losing heat in three ways, (a) by direct radiation through the open mouth, (b) by inhalation of cool air

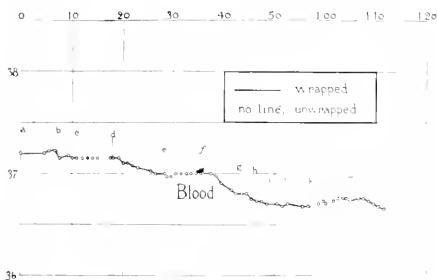


Fig. 3.—Blood temperature control. *a*, electric light is reflected on thermometer, *b*, light removed; *c*, unwrapped; *d*, wrapped; *e*, unwrapped, fan on; *f*, fan off, wrapped; *g*, muscles of subject under tension; *h*, subject feels warm; *i*, subject sits comfortably, skin seems flushed; *j*, subject feels cold; *k*, unwrapped, cold towels to back; *l*, fresh cold wet towels to back; *m*, dried and wrapped.

and exhalation of warm air in respiration, and (c) by conduction down a thermal gradient established through the air in the mouth, and especially along the metal applicator, to the cold room outside. From what has been said above it is evident that the rate of heat loss by (a) radiation, and (c) conduction was kept virtually constant throughout each experiment. In all the experiments cited below (b) respiration was carefully controlled—rate by breathing in time with a metronome, and depth by the use of a thoracic and an abdominal pneumograph writing on a smoked drum in sight of the subject.

Finally, the effects of air currents and eddies in the experimental room must be considered. Although the sites of application of the thermopiles were protected from the direct draft of the fan, minor currents and eddies were necessarily set up in the small closed room and the direct cooling effect of these upon the exposed skin could not be eliminated. This direct cooling, as closely as we have been able to estimate it, probably amounted usually to between one-third and one-half of the observed skin temperature fall. The curves should be

studied with this correction in mind. On the other hand it is obvious that in experiments in which the mouth was closed and the applicator upon the pharyngeal wall, currents in the room could not have entered at all into the depression of mucous membrane temperature. Similarly with the applicator in the nasal cavity and nose breathing, or on the palate or pharyngeal wall, even with the mouth open, direct cooling by air currents in the room could have entered but slightly or not at all into the observed mucous membrane temperature fall. The effect of the air currents, then, has been to make appear less striking in comparison with those of the skin the vasoconstrictor reflexes of the mucous membranes with chilling of the body surface.

The thermopiles when applied under the conditions described do not record precisely the absolute temperatures of the surfaces as they would be in the absence of the thermopiles. However, we believe that this method does afford an accurate and sensitive means of recording superficial temperature changes, and, through these, states of vasomotor tone, and it is with these that we are concerned. Measurement of the absolute mucous membrane temperature, if desired, could be effected by a modification of the method used for the skin by Benedict, Miles and Johnson (1919).

**Control Experiments:** In order to determine the validity of mucous membrane temperature as a criterion of vasomotor tone it was necessary to study changes effected in respiration, blood pressure and blood temperature by the conditions of our experiments. Without going into detail, the results of these control observations may be briefly summarized.

Chilling the body surface has, both in our controls and in animal experiments (Ansiaux 1889, p. 569) increased the volume of respiratory change, and this in turn often depressed mucous membrane temperature. It was for this reason that control of respiration by metronome and pneumographs was necessary.

Blood pressure was not, in the two trials made, significantly altered by chilling. Animal experiments have shown an initial rise, followed, in instances of extreme chilling with depression of blood temperature, by a progressive fall (Marchand, 1908, p. 125, and Ansiaux, 1889). The depression of superficial temperature observed with cutaneous chilling was therefore not the result of lowered blood pressure.

Blood temperature, as has been said, underwent only slight alterations during the course of our experiments. So efficient is the heat regulating mechanism in man, indeed, that during chilling the blood temperature rose very slightly, to undergo a slight fall on cessation of chilling. (Fig. 3.) Liebermeister (1860) found essentially similar changes in axillary temperature with chilling of the skin.

*(To be continued.)*

# UNSUCCESSFUL RESULT FOLLOWING TRANSFUSION WITH IMMUNIZED BLOOD IN A CASE OF INFECTIOUS ENDOCARDITIS

By LOUIS A. LEVISON, M.D., TOLEDO, OHIO

THE treatment of various infectious processes by the transfusion of blood taken from donors who have been immunized against the bacterial agent involved has introduced a new therapeutic procedure that is now on trial. Within recent years there have been several reports of such cases by various observers, but the scarcity of these instances and the variable circumstances under which they have been managed renders a conclusion in regard to their value very difficult. Conclusions based upon individual case reports are notoriously unreliable.

An instance of a successful result from the transfusion of immunized blood was reported by Little<sup>1</sup> who cites the case of a girl of eleven with epidemic influenza complicated by laryngitis, pleurisy, suppurative glossitis and finally a general septicopyemia with the isolation of a staphylococcus and an unidentified bacillus from the blood. A vaccine was made and a donor immunized by giving an injection prior to the last two transfusions. In all four transfusions were given. The septic temperature disappeared and the patient recovered. It was the belief of Little that this therapeutic procedure was life saving in this instance.

## CASE REPORT

W. R., age twenty-one, occupation college student, unmarried.

*Family History.*—Father and mother both living and well. Nothing unusual in the history of relatives or antecedents.

*Personal History.*—Diphtheria at age of two. Patient is a third year student in the University of Michigan and has not been sick during his period of school. In 1918, when examined for military service was found to have a loud systolic murmur at the apex of the heart and was summarily rejected from duty. This murmur was not known to him before and so far as he or the family knew, there had been no trouble in any way from cardiac trouble. Patient did not pursue any strenuous athletic exercises, but ordinary activities were easily carried out.

*Present Illness.*—The present illness began in March, 1920. There were evidences of a sore throat about this time but no definite attack of acute tonsillitis. The patient was examined several times during this month at the medical clinic of the University of Michigan where a heart lesion and fever were discovered on account of which he was requested to remain in bed for short intervals of time. However the fever was not a continuous one so that he was permitted to leave his bed and resume his student activities. Three times during the month of March there were times when he was confined to his bed with fever for short periods, resuming his work in the intervals. About the first of April the fever became more persistent and he was sent home from school and did not return. Since that time, he has been confined to his bed continuously. Fever was a constant symptom varying from 99 $\frac{1}{2}$  to 103 $\frac{1}{2}$ . There was no regularity in the temperature curve but the afternoon temperature was generally the higher. The pulse ranged from 100 to 120 and the respiratory rate was not increased. There was no evidence of cardiac decompensation such as edema, dyspnea or cough. Patient was fairly comfortable while lying quietly in bed. There were no pulmonary

<sup>1</sup>Little: Jour. Am. Med. Assn., LXXIV, 734.

symptoms, and the gastrointestinal tract did not give rise to any unusual trouble, except occasional distress following his food. There were no urinary symptoms. Later certain complications ensued.

*Examination.*—The patient, at the outset, was of good size, weighing 180 pounds. Emaciation was gradual and steady. Nothing unusual was noted as to the condition of the bones, joints, or lymph glands. The cheeks were flushed. The skin showed a gradually increased pallor with the progress of the disease. There was no delirium. Speech was normal. The tonsils were large, spongy and showed yellow deposits filling some of the crypts. This condition persisted throughout the course of the disease. Teeth were good, throat showed a chronic pharyngitis. Neck: Thyroid not enlarged. A distinct pulsation was felt in suprasternal notch, coinciding with the systole of the heart. The carotid pulsation in the neck was throbbing and violent and could be seen from a considerable distance.

*Heart.*—Inspection showed a waving apex impulse extending to the anterior axillary line. No thrill on palpation. Percussion showed heart dullness, increased transversely, and also a slight widening of the aortic dullness. There was a loud systolic murmur to be heard all over the precordium and also in the left axilla, left back and the first and second inter-spaces of either side. There was also a diastolic murmur heard at the base of the heart. The first sound was distinctly heard, but was gradually replaced by the systolic murmur. The second sound at the base was faintly heard. The radial pulse was equal on the two sides. Blood pressure at the outset was 120/0. Later the systolic pressure dropped gradually to 90 and a short time before the end to 80. There could be heard at all times a distinct note on auscultation over the brachial artery during diastole. A capillary pulsation was noted.

*Abdomen.*—Liver and spleen were not large and could not be palpated. There was moderate tenderness over the epigastrium during the periods when he had gastric distress, but no other abdominal findings. The genitourinary apparatus was negative.

*Laboratory Findings.*—The urine was dark yellow in color, acid specific gravity ranging from 1016 to 1022, albumin present in small amounts at intervals, no sugar. Granular casts were occasionally seen with a few red cells and leucocytes.

*Blood.*—Red count decreased gradually from 4,100,000 downward, but the figure varied considerable on account of repeated transfusions. The white count before transfusion varied from 18,000 to 22,000. The percentage of polymorphs was 88 per cent. Blood culture showed a pure growth of streptococcus viridans. (Dr. Pamment). Blood group II. Wassermann reaction negative.

*Course of Disease.*—During the course of the disease, which extended from April until July inclusive, there were several complications which appeared. Towards the latter part of June the left hip became very much swollen, apparently fluctuating, and excruciatingly painful. Morphine was required to allay this pain. It was thought that pus was present and repeated aspirations were made. However, no fluid was found excepting small amounts of blood. It is probable that this condition was caused by a hemorrhage into the hip joint and surrounding tissue. Later there was an embolus in the left lung with the sudden onset of cough, bloody sputum and an increased respiratory rate. This cough and bloody sputum persisted until the end.

*Treatment.*—In the absence of any specific treatment for infectious endocarditis, symptomatic measures were employed throughout the illness. Injections of sodium cacodylate were without apparent effect. Intravenous injections of electargol were employed also without apparent result. It was decided after the isolation of the streptococcus from the blood to prepare a vaccine from this organism, immunize a donor (Group II) and give the patient repeated blood transfusions from this immunized blood. A donor was selected in the person of a healthy, stout, maternal uncle, weighing 200 pounds. He was given repeated injections of vaccine prior to the first transfusion and continued throughout at intervals of four days. Four transfusions were done by the citrate method employing 500 to 600 c.c. of blood at each injection. In all 2200 c.c. of blood were transfused within a period of five weeks. Following the first transfusion there was a moderately severe reaction with a chill and rise in fever, but these reactions practically disappeared after the later ones. The

result of the transfusions was to increase the hemoglobin content of the blood, raise the number of red cells, lower the number of leucocytes and decrease the percentage of polymorphonuclear cells. The strength of the patient was maintained by this procedure to a marked degree. However there was no definite change in temperature curve following any of the transfusions. At no time did the temperature remain normal for the duration of even one day. The pulse rate was not reduced. The hemorrhage in the hip joint and the embolism into the lung occurred during the period of time in which the transfusions were being employed, but did not directly follow any of them. On July 8 the temperature dropped to normal, but with it there was an increase of the pulse rate to 140. Patient became weaker and the pulse irregular. Exitus occurred on July 10 with the patient comatose and the heart in fibrillation.

My suggestion that immunized blood transfusions be employed in this instance was concurred in by Dr. C. F. Hoover of Cleveland, who stated that this procedure was a logical one theoretically. In view of the desperate nature of the patient's illness and the entire absence of known therapeutic aid, the patient was very willing to have the experiment attempted. The fact that the donor received the vaccine five times before the first transfusion with a reaction following each injection consisting of a temperature rise, chill, malaise, and an increase in leucocytes is evidence that the donor did respond to the vaccine or in other words was "immunized." The vaccine injections were continued in the case of the donor throughout the intervals between the transfusions. Whatever antibodies might have been produced by this procedure were introduced into the patient in the course of the subsequent transfusions. The experiment was carefully observed and it can be said that beyond the maintenance of the patient's strength and the relief of the anemia that the transfusions were of no avail.

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## JAMES THEORY OF THE EMOTIONS IN RELATION TO THE ADRENAL GLANDS

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BY CHARLES E. KIELEY, CINCINNATI, OHIO

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JAMES postulates that we cannot experience an emotion unless we also experience the physical changes that habitually accompany it. He states his postulate even so boldly as: "We are afraid because we run" rather than "We run because we are afraid." Cannon and his collaborators attempted to show that the physical concomitants of fear were produced through psychic stimulation of the adrenal glands. Accepting both theories it follows that a blocking of the adrenal hyperactivity should prevent the experience of fear. Biedle states that there is but one known substance which neutralizes adrenalin physiologically at all points. This is apocodeine, a drug bearing the same relation to codeine as apomorphine to morphine but not exhibiting the same pharmacologic action as apomorphine. Apocodeine was first prepared by Matthieson and Wright in 1870. It was admitted to the British Pharmacopeia of 1890 as a hypodermic cathartic, but is now almost obsolete, though recommended lately for various intestinal conditions. The subjects for the present therapeutic tests were both psychasthenies whose most prominent symptom was fear.

Miss X is now thirty. She came under my care in 1916. The chief complaint was fear of death. Long and persistent questioning finally showed the fear to have its basis in her religious attitude toward her erotic desires. At five she recalls masturbation by contact with furniture. At eight, and again at thirteen, she submitted to sexual relations by an older cousin, but denies physical indulgence of any kind since then. She is a fervent member of a religion which teaches that to entertain sexual fantasies is as great a sin as physical indulgence and the guilty will be eternally punished. This is the psychic mechanism which determines the fear of death.

Physical examination of the girl has been exceptionally minute and frequently repeated without showing any very definite organic disease. She has always a marked tachycardia, the pulse ranging from 90 to 120 while sitting. She has polakuria but this is voluntary as she experiences greater eroticism if she allows her bladder to become even moderately full. There is an unexplained inequality of the pupils. Gynecologic examination has shown a hypertrophy of the labia minora. Cystoscopy has twice been negative. Innumerable cardiac examinations have shown no organic lesion. Every conceivable form of therapy has been tried—sedative drugs, rest cure, suggestion and glandular extracts. Suspicious teeth have been removed and (without my advice) a curettage performed. Not the least effect has been noted. Finally I have given her hypodermically from 1 to 2 c.c. of 1 per cent apocodeine solution every other day until 20 injections were reached. She did not experience even the slightest temporary alleviation of her anxiety.

Mr. Y is thirty-five. His past history shows as significant for psychoneurotic disease, only a skull fracture thirteen years ago, which has left him deaf in both ears. During the influenza epidemic he was advised to drink whiskey for prophylaxis. For three months he had one or two drinks a day and dates his fears from that time. His phobia is mainly that of committing suicide. He is well satisfied with life and has no desire for death but is obsessed with a fear of self-destruction. This man has been given 15 injections of 1 per cent apocodeine ranging from 1 to 2 c.c. As in the previous case there has never been any sense of relief from the fear.

#### CONCLUSION

Granting that Cannon's contention in regard to the bodily effects of fear is correct, the two experiments presented here indicate that an emotion can be experienced independently of the physical changes which habitually accompany it.

# A PRELIMINARY REPORT ON BLOOD COAGULATION\*

By E. C. MASON, M.D., CINCINNATI, OHIO

## INTRODUCTION

THE generally accepted interpretation of blood coagulation makes it necessary to use some eight or nine terms, such as, prothrombin, thrombin, proantithrombin, antithrombin, thrombokinase, etc. These terms are used only to name conditions and furnish no light on the true nature of the clotting. Most, if not all, of these terms are given to substances that have been isolated through laboratory methods which furnish products never present at the time of normal clotting.

Several years ago, while studying the nature of the clot formed in human and cow's milk, I was impressed by the action exerted by certain protective colloids. I observed that such colloids made the casein more resistant to precipitating agents, and also that the clot, formed in their presence, was of a different nature. Such protective colloid substances have been used for years in the modification of cow's milk for infants;<sup>1</sup> however, the method of their action was not completely understood. Alexander,<sup>2</sup> in his splendid work on this subject, has demonstrated that the lactalbumin stabilizes and protects casein from coagulation by acid and rennin. With a knowledge of these results, and realizing that the process of milk coagulation is, in many respects, similar to the process of blood coagulation, I conceived the idea that protective colloids probably played a part in blood coagulation. I have been making a study of blood coagulation, with this in mind, and have briefly outlined my observations in the following paragraphs.

## ANALYSIS OF THE CLOTTING PROCESS

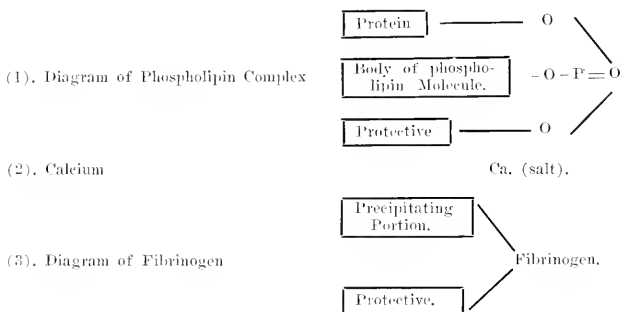
The fewer the terms used, the less chance for confusion. Therefore I have restricted myself to three. They are; fibrinogen, calcium and phospholipin complex. Of the three factors mentioned, it is certain that fibrinogen and calcium are present in the plasma of the circulating blood. The question arises as to whether or not the phospholipin complex is present, and if present, is it in some inactive form. There is convincing evidence<sup>3</sup> that such compounds are present, in the circulating blood, in the inactive form.

The present plan considers that after the protective influence is removed from the phospholipin complex, coagulation is the result of the combination of the three substances in the diagram; that is, phospholipin, calcium and the precipitating portion of fibrinogen, and the final clot contains these portions. The calcium (salt) probably binds to the phosphorus in place of the protective portion, and the other bond of calcium binds the fibrinogen portion.

I present the following scheme, which I believe will enable one to understand

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the process. I have used one phosphorus atom as the nucleus, but considering the usual formula for this type of compounds, I should doubtless have used more than one.



#### EVIDENCE THAT FIBRINOGEN SPLITS IN THE PROCESS OF CLOTTING

The main question seems to be, whether fibrin pre-exists in the circulating plasma maintained in solution by other proteins (colloids), and this equilibrium disturbed at the time of coagulation; or is a soluble protein, fibrinogen, in *some unknown manner* converted at the time of coagulation to an "insoluble" product, fibrin. The last view is the one generally accepted. However, the view was strongly attacked by Wooldridge, and the objections which he advanced apparently have not been answered.

Throughout our discussion fibrinogen is spoken of as made up of two portions, (1) the protective portion and (2) the portion capable of being "precipitated." To illustrate the two portions represented in fibrinogen, I have selected, for the sake of simplicity, a fat such as stearin (glyceryl-stearate), the characteristic reactions of which are common to *fibrinogen* and *cascinogen*.

*First.* The acid treatment of each yields the "insoluble" hydrogen precipitate.

*Second.* The sodium salt of each is "soluble." It is also possessed of a high hydration capacity.

*Third.* The calcium salt of each is "insoluble," and this compound has a low hydration capacity.

*Fourth.* If stearin (glyceryl-stearate) is treated with a calcium salt, no "precipitation" of calcium stearate occurs, but if first treated with sodium hydrate, and then with a calcium salt, the calcium stearate is formed. This is also true of fibrinogen.

*Fifth.* If compounds such as glyceryl-stearate are treated with sodium chloride, there is no apparent reaction, but if the hydrogen stearate (stearic acid) is treated with sodium chloride, there is an interchange with the formation of some sodium stearate, which is "soluble."

*Sixth.* The function of the catalytic agent, or "enzyme," is to split each into the two portions.



The work of Hammarsten,<sup>4</sup> Schmiedeberg,<sup>5</sup> Henbner<sup>6</sup> and others, shows that in the transformation of fibrinogen to fibrin a globulin is split off. This is termed *fibrino-globulin* by Hammarsten and serum-globulin by others.

*Evidence that compounds such as give rise to "thrombin," remain in the tissues (including blood as a tissue) in the protected form, and if such protection is removed they combine in the formation of the final clot.*

The chemical nature of the substance which is active in promoting the fibrin formation (thrombin), has been studied by various investigators. Halliburton<sup>7</sup> and Pekelharing<sup>8</sup> isolated a body from Schmidt's extract which gave proteid reactions, and resembled the globulins in many particulars. At first they classified it as a globulin,<sup>9</sup> subsequently both recognized that the substance was not a globulin and classed it as a nucleoprotein.<sup>10</sup> Pekelharing considered that this nucleoprotein in combination with calcium constituted "thrombin," and stated that it could be formed not only from the nucleoproteins of the plasma or serum, but also from the nucleoproteins in the cells of the thymus, testicle, and other glands, by digesting these with calcium chloride, the excess of the calcium salt being afterwards dialyzed off. According to Pekelharing, this combination does not give up the calcium when dialyzed, nor is the combination broken up by soluble oxalates, although if these are present from the first, they may prevent the original union. Howell, after much careful work on this subject, has stated the following:<sup>11</sup> "My own opinion is that thrombin is a protein or protein derivative."

It appears evident that the function of the calcium, in the process of blood coagulation, is to activate the thrombin, and that there is something present in the normal blood which prevents this action of calcium. For the want of a better name I have termed this the *protective portion*. Various substances, including tissue extract and cephalin, are capable of removing this protective portion, and as a result, calcium activates the thrombin when clotting takes place.<sup>12</sup> "It would seem quite possible or probable that these fibrin-aggregates represent in fact a real physiochemical combination between fibrinogen and thrombin."

Rettger<sup>13</sup> and Howell<sup>14</sup> have definitely shown that there is a combination of the thrombin and fibrinogen in the clotting process, demonstrating that definite amounts of thrombin yield definite amounts of fibrin. Wooldridge's<sup>15</sup> analyses also show that fibrin contains a large percentage of phospholipins. Such phospholipins are not taken into consideration in the ordinary analysis of fibrin, as they are removed from the normal clot by extraction with ether or alcohol.

Howell<sup>16</sup> has shown that the active principle of tissue extract is a phospholipin protein compound, and, as indicated by the work of Pekelharing, tissue extract when treated with a calcium salt will act as thrombin. Therefore, with these two points in mind, I have selected the complex which is presented in the diagram of the clotting process. However, the exact chemical formula need not be seriously considered, for that could vary greatly and the working plan still hold.

Apparently the normal  $\text{c}^2\text{c}^4$  is the union of the three substances, and at present I am engaged in finding out the effect produced on the hydration capacity of this system, as the result of varying the amount of each present.

## SUBSTANCES WHICH RETARD THE COAGULATION OF BLOOD

Substances retard the coagulation of blood by doing the following:

*First.* Increasing the protective portion of the phospholipin complex (anti-thrombin).

*Second.* Preventing the splitting off of the protective portion of either the phospholipin complex or fibrinogen.

*Third.* By removing any one of the three factors.

Any condition which will increase the amount of the protective colloid (anti-thrombin), will retard the coagulation. *Such conditions are realized in the autolysis of tissues of the body, including the white cells.* Evidence for this statement will be found in the observations of the following men: Doyon<sup>17</sup>, Wells,<sup>18</sup> Conrad,<sup>19</sup> Whipple,<sup>20</sup> Erben,<sup>21</sup> Pfeiffer,<sup>22</sup> Minot and Denny,<sup>23</sup> Opie,<sup>24</sup> Bell,<sup>25</sup> Dienst<sup>26</sup> and Dochez.<sup>27</sup>

## SUBSTANCES WHICH ACCELERATE THE COAGULATION OF BLOOD

Substances accelerate the coagulation of blood by doing the following:

*First.* By removing the protective portion of the phospholipin complex.

*Second.* By contributing reactive material, (one of the three factors presented in the diagram of coagulation, the degree of acceleration depending on the factor increased).

The following substances accelerate the coagulation of blood by one of the two methods just mentioned:

(1). Glass vessels, finely divided glass, glass wool, clay filters, absorbent cotton, etc. (2). Certain synthetic colloids. (3). Electric current. (4). Injured tissue and tissue extracts. (5). Cephalin. (6). Carbon dioxide and acetic acid in the negative phase of coagulation produced by peptone injection.

These substances all have one point in common, being *electro-negative*. This property apparently plays a very active part in their reaction, for if the charge is reversed, as in the synthetic colloids prepared by Grimaux,<sup>28</sup> they lose their activity. Again as in the case of the electric current,<sup>29</sup> coagulation takes place at the pole which accumulates negative charges.

*Apparently the protective portion of the phospholipin complex is either neutralized, or its place satisfied by reacting with electro-negative substances.*

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## AN ANALYSIS OF ONE HUNDRED POSTMORTEM EXAMINATIONS IN SIAM\*

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**M**EDICAL literature has so few references to conditions in Siam that I believe statistics of 100 consecutive postmortems are of sufficient clinical and pathologic interest to merit publication. These were the first made by me in Bangkok and were performed in Siriraj Hospital between August 1, 1919, and June 10, 1920. Twenty-two additional ones were made by other members of the laboratory staff, but are not here included because the records are incomplete. During this period there were 270 deaths in the hospital, which gives a total of 122 postmortems in 270 cases, or 45 per cent. This percentage compares very favorably with that in the United States, where, judging from my own knowledge and from medical literature appearing since I left, things are not entirely satisfactory.

It has been highly gratifying to me during the year to note the number of articles calling attention to undesirable conditions regarding the number of postmortems and the teaching of pathology therefrom. Discussion of this means improvement. If real efforts are made to locate the cause it will doubtless be found that in most hospitals the small number of postmortems is due to remediable conditions. Then let the remedy be applied. For there can be no better place than the postmortem table for the student to learn pathology and for the clinician to check up his diagnoses. And the physician in charge of the case should attend with his Senior students who have studied the patient in the hospital. In my opinion the attending physician should do the greater part of the teaching at this time. The pathologist should point out the morbid anatomy of the lesions and in the light of these the physician can

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discuss with the students the physical signs and the symptoms that had previously been noted. The student must not come away from a postmortem remembering simply that he saw a diseased heart, or a pneumonic lung, or a cirrhotic liver; in addition he must place these facts as the final points of knowledge regarding an individual case of a certain disease. The physician who has been studying the case can aid the students in properly placing these facts; the pathologist can do relatively little. Sending to the postmortem room the history, with or without the interne who wrote it, is an aid but it is an extremely poor substitute for the physician who is teaching the students medicine. If this team work by pathologist and clinician were properly developed, both, as well as the students, would benefit greatly thereby. Each would learn from the other and there would be less petty fault-finding and criticism about each other's work.

Of the 100 cases here recorded, 64 were males, 36 females. Most were Siamese, but Chinese were included.

The ages ranged from stillborn to 67 years. In ten-year periods they were as follows:

Under 10 years .....	11
From 10 to 19 years .....	5
" 20 " 29 " .....	23
" 30 " 39 " .....	17
" 40 " 49 " .....	10
" 50 " 59 " .....	17
" 60 " 67 " .....	5
Age not recorded .....	12
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100	

The lesions in the cases as recorded in the gross diagnoses varied in number from three to fourteen. The primary or chief cause of death as determined from these and the clinical course of the disease was the following:

Abscess of liver, amebic .....	1
Abscess of pelvis .....	3
Appendicitis, gangrenous .....	1
Atelectasis of lungs .....	1
Bronchial asthma .....	1
Bronchopneumonia .....	5
Calculus of bladder (cystitis) .....	2
Carcinoma of liver .....	1
Carcinoma of stomach .....	1
Carcinoma of uterus .....	1
Cholera .....	3
Cholecystitis, suppurative .....	1
Cirrhosis of liver, atrophic .....	5
Dysentery, amebic .....	17
Dysentery, type doubtful .....	3
Endocarditis, acute .....	2
Endocarditis, chronic .....	1
Endometritis, puerperal .....	3

Edema of brain .....	1
Fatty degeneration of heart .....	3
Fracture of skull .....	2
Gangrene of lung .....	1
Gastroenteritis, chronic .....	1
Gunshot wound of abdomen .....	1
Heat exhaustion .....	1
Hypernephroma of kidney .....	1
Influenza-pneumonia .....	2
Knife wounds .....	2
Lobar pneumonia .....	1
Lymphosarcoma (duodenum) .....	1
Malformation of heart and vessels .....	1
Meningitis (tuberculous) .....	1
Mercurial poisoning .....	1
Myocarditis (chronic) .....	3
Nephritis (suppurative) .....	2
Opium poisoning .....	1
Rupture of uterus .....	1
Sarcoma of (Liver) .....	1
Septicemia .....	2
Syphilis .....	2
Suffocation (newborn) .....	1
Tuberculosis (lungs) .....	10
Tuberculosis (hip) .....	1
Typhoid fever .....	3
Uncinariasis .....	1

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 100

This table serves to show the wide range of cases making up the series and also brings out one point worthy of special mention, namely, that 27 deaths (more than one-fourth the total) were due to diseases of the intestines. These were:

Dysentery (colitis) .....	20
Cholera .....	3
Typhoid fever .....	3
Uncinariasis .....	1

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 27

This emphasizes a well-known fact, namely, the high mortality rate in tropical countries from intestinal diseases. These are due almost entirely to bacterial or parasitic contamination, primarily of soil or water, and secondarily of the food supply. The almost constant high temperature of such countries favors the continuous growth of animal parasites and bacteria which in colder climates are checked or for a time prohibited by periodic freezing of the ground. This, aided by common methods of soil fertilization (by human excrement) furnishes a prolific source of disease. It is a point constantly to be emphasized about the tropics to medical students and physicians, especially those engaged in Public Health service. The latter have particularly difficult conditions with which to deal in the prevention of disease.

Some of the findings in these cases, as will be noted later, belong to the rarer lesions but most of the cases were of the more common ones that are especially valuable to students. I believe that we as teachers too often spend on rare cases time that could be much better employed on the fundamentals of common ones. It is true that unusual cases are always deeply interesting to the teacher and are of great value in medical literature. They must also be brought to the attention of the student but of the time of the latter they require only a minimum. Many of the conditions here recorded were found just at the right time to illustrate topics of the lecture course and thus to quite a degree helped atone for the scarcity of museum specimens. In none of my many series of 100 postmortems has there been one with more cases of real teaching value.

Before taking up in detail some of the points brought out by this series, I wish to give a summary of the lesions in the chief organs. This will also give an idea of the frequency with which the various organs were diseased. Of course a series of 100 cases is not large enough from which to draw lasting conclusions as to frequency of a certain condition. It will, however, give a general idea of the findings here as compared with those in the United States.

#### HEART:

Aneurism,	1
Atrophy,	2
Brown atrophy,	1
Dilatation	{ Left ventricle, 19 Right ventricle, 13 Left auricle, 3 Right auricle, 13
Endocarditis	{ Mitral { acute, 3 { chronic, 1 Aortic { acute, 1 { chronic, 2 Mural, 1
Degeneration	{ Fatty, 18 { Parenchymatous, 31
Hypertrophy	{ Left ventricle, 5 { Right ventricle, 5
Malformation,	1
Malposition,	1
Myocarditis, fibrous,	21
Patulous foramen ovale,	7
Tumor, secondary,	1

#### ARTERIES:

Absence of pulmonary,	1
Aneurism of aorta,	1
Arteriosclerosis	{ General, 1 Aorta, 10 Coronary arteries, 3 Syphilitic { Aorta, 3 { Pulmonary, 1

#### LUNGS:

Abscess,	3
Atelectasis,	7
Bronchiectasis,	1
Bronchitis	{ Acute, 17 { Chronic, 3
Congestion,	47
Edema,	30

Emphysema	{	Vesicular, 43
		Interstitial, 2
		Interlobular, 1
Gangrene, 1		
Infarct, 3		
Malformation, 3		
	{	Lobar, 1
Pneumonia	{	Broncho {
		Left, 9
		Right, 7
		Chronic interstitial, 2
	{	Active {
		Left, 10
		Right, 7
		Healed {
		Left, 6
		Right, 7
Tumor, secondary, 1		

## PLEURAE:

Hemothorax, 3		
Hydrothorax, left, 4; right, 6		
	{	Acute {
		left, nonsuppurative, 2
		right, nonsuppurative, 4
		right, suppurative, 1
	{	Chronic adhesive {
		left, 25
		right, 27
Pneumothorax, 2		

## SPLEEN:

Accessory, 14	
Absence, 1	
Atrophy, 5	
Congestion, 23	
Chr. capsulitis, 7	
Chr. splenitis, 7	
Enlargement, 27	
Infarction, 1	
Pigmentation, 7	
Softening, 8	
Tuberculosis, 2	

## KIDNEYS:

Congestion, 17	
Hydronephrosis, 1	
Hypernephroma, 1	
Malformation, 1	
Malposition, 2	
Nephrolithiasis, 1	
	{
Nephritis	{
	Parenchymatous {
	acute, 9
	chronic, 8
	Diffuse, chronic, 6
	Interstitial, chronic, 10
	Suppurative, acute, 3
Parenchymatous degeneration, 25	
Tuberculosis, 2	

## STOMACH:

Carcinoma, 1	
Carcinomatous ulcer, 1	
Congestion, 8	
Gastritis, acute, 1; chronic, 7	
Ulcer, 2	

## LIVER:

Abscess, amebic, 3	
Cirrhosis—atrophy, 5; congestive, 8; syphilitic, 1	
Congestion, 39	
Fatty infiltration, 14	
Malformation, 1	
Pigmentation, 4	

Parenchymatous degeneration, 27

Tuberculosis, 2

Tumor, angioma, 1; carcinoma, 1; Hypernephroma, 1; sarcoma, 2

#### INTESTINE:

Duodenum, ulcer, 2; lymphosarcoma, 1

Small intestine	{	Enteritis, catarrhal, 8; follicular, 3; ulcerative, 4
		Stabwound, 1
		Tuberculosis, 6
		Lipoma, 1

Typhoid lesions, 3

Colon	{	Colitis, amebic, 17; catarrhal, 4; ulcerative, 6
		Tuberculosis, 4
		Tumor, 1
		Syphilis, 1

Stenosis, 1

Rectum, gangrene, 1

#### PERITONEUM:

Abscess, appendiceal, 1; pelvic, 2

Ascites, 16

Carcinoma, 2

Peritonitis, acute, 11; chronic adhesive, 8

Tuberculosis, 1

#### MISCELLANEOUS:

Jaundice, 4

Transposition of viscera, 1

Leukoderma, 1

Fracture of skull, 2

Fracture of humerus, 1

Ascariasis, 2

Uncinariasis, 1

Tapeworm, 1

A very common condition here is vesicular emphysema of the lungs. This was a feature I noticed from the beginning because of its much greater frequency than I had found in the United States. This diagnosis, given in the table as 43 cases, could have been given a greater number of times if note had been made of its evident presence in lungs that had other lesions, as pneumonia, tuberculosis, or congestion and edema. Minor degrees were not recorded as I have come to regard the condition as almost constant here in adults. In many it is of an advanced degree. The type is the essential, or large-lunged, emphysema. Why this is true I do not know. Castellani and Chalmers say that "emphysema and asthma are fairly common in the tropics." I am informed that asthma is quite frequent in this country but do not believe it is so universal as emphysema seems to be. The condition is an interesting one for which I shall seek further explanation.

Some of the other lesions are less frequent than we are accustomed to find them in the United States. The presence of gallstones was noted in two cases; in both they were small and few in number, not at all like the dozens or hundreds as we find them in not a few bodies.

Sclerosis of arteries is less common here. In only one of the cases was the diagnosis of general arteriosclerosis justified, although in several there was sclerosis of certain arteries, as the aorta, coronaries, or renal. In only one case was there extensive involvement of the aorta with large yellow firm patches, a few calcareous. With the extensive amount of salts in the water here and the fre-



quency of bladder calculi, it might be thought that calcareous infiltration of damaged vessels would be frequent. From other observations regarding this condition, I believe it would, were the vessels damaged as so often found in elderly persons in the States.

The rarity of gross syphilitic lesions in spite of the supposed great prevalence of that disease has been a striking point. In the absence of Wassermann tests in the hospital, the number of cases among the one hundred cannot be stated but the postmortem manifestations were not indicative of many. Of the lesions tabulated under vessels and liver, most were in one case, a woman of thirty-six years. This body furnished the syphilitic cirrhosis of liver, arteritis of pulmonary artery and one of the instances of aortic involvement, and the aneurism of the aorta.

Another condition that theoretically should be very often found proved not to be so. This is malarial pigmentation of the liver and spleen, with enlargement of the latter. The table shows 7 cases of pigmentation of the spleen and 4 of the liver. The cases of enlargement of the spleen as tabulated, 27 in number, were mainly those of infectious conditions or congestion due to other well defined diseases. The instances of pigmentation were mostly in moderate-sized organs instead of notably large ones. Not all the spleens were examined microscopically and it may be that more of them were pigmented than the size and color would indicate, but I am speaking now of gross appearances. This is one of the many problems to be investigated in further series of cases. A point to be remembered in this connection is that the large majority of cases in the series were from Bangkok, in which malaria is not nearly so frequent as in the jungle regions further North.

Malformations were not numerous in this series. The most notable was the one with an opening in the interventricular septum of the heart with absence of the pulmonary artery and spleen. These, with accompanying transposition of viscera, made the case a most unusual one. It has been published in the *Journal of the American Medical Association*. This child also had a four-lobed lung on each side; in two other bodies the left lung had partial separation into three lobes. One kidney was moderately changed in shape and was also out of position.

One newborn child had malformations of the external genitals making it worthy of the term hermaphroditism, although it seemed fairly certain a male. The scrotum resembled labia and the rudimentary penis had complete hypospadias. The testicles were found in the abdominal cavity near the brim of the pelvis.

An interesting malformation of the bladder was found in a woman of sixty-five years. This was a diverticulum extending from the right lateral portion. It began as an opening 1.5 cm. in diameter and gradually increased in size to its termination, being 6 cm. long. This sac was entirely empty. The absence of calcareous material is of interest because the pouch would seem to have furnished admirable anatomical conditions for the formation of calculi. Of course women are less liable to this condition than are men.

Finally a peculiar malformation was the condition of the great toes in a man of unknown age. Each was short, only half as long as the second toe of

each foot. It may be said in passing that supernumerary little fingers and thumbs are not of great rarity in Siam, though none of the bodies in this series showed them. Supernumerary mammary glands are also fairly frequent, three cases being in the Obstetric Department of the hospital at one time.

#### TUBERCULOSIS

Death was attributed to this in 11 cases, 10 of the lungs and one of the hip. In some of the pulmonary cases tissue destruction was very great, cavities occupying the whole of an upper lobe being found. In spite of this, however, the general statement can be made that the process does not appear so widespread, so active, so broadly destructive as in the average case in the United States. The impression gained is that the tissue destruction is spreading slowly. The cavities as a rule have quite smooth walls and many have considerable fibrous tissue in them. Around the cavities may be found small tubercles, but rarely any extensive areas of necrosis. In some cases with a cavity of 2 cm. in diameter, no tubercles are seen near it. In the lower lobes of lungs with extensive cavities in the upper, are often areas of conglomerate tubercles but with very little necrosis in the center. Even in the upper part of such lobes, with small cavities present, necrosis does not appear prominent or active. In cases without extensive destruction (with death due to other causes) I have found cavities of 1 or 2 cm. in size in a lung, with no tubercles whatever around them: in one instance, we examined spreads from the wall, finding it loaded with tubercle bacilli, before I was finally convinced that we were not dealing with an abscess.

Many of these cases had no involvement of spleen, liver, lymphnodes or intestine. Some had tuberculous ulcers of the intestines with numerous tubercles in the serous coat. Of course the disease destroys tissue and causes death but the general appearance is that the tissue has been putting up a good resistance. The duration of the cases I do not know and it may be the clinical history (if it could be obtained accurately) would negative this supposition based on the appearance of the lungs. Whether the cases were of slow progress and long duration or not, the fact remains that the disease was responsible for 11 of the 100 deaths. It should be added that in 11 other bodies there was healed tuberculosis in one or both lungs. This adds to the total number of infections. So far as climate is concerned, Bangkok would seem to be one of the worst for tuberculous persons. It is only four feet above the sea level and during half of the year the air is extremely moist.

#### INFLUENZA AND PNEUMONIA

Two deaths were attributed to influenza and its complicating lung lesions. One of these clinically was influenza with a duration of 10 days; the lungs were typically like those of the thousands of cases coming to postmortem during the great epidemic of 1918. The other patient was brought to the hospital unconscious and died in 20 hours with no clinical history obtainable. The lung lesion was in a fairly early stage, but I am convinced it was of the same type. Cases of this disease, whatever it should be called (we are calling it influenza),

are occurring here from time to time. In addition to the two cases here reported, one typical case with pneumonia came to postmortem recently at the Chulalongkorn hospital. I think there is no doubt that some cases of common cold, of bronchitis, and of bronchopneumonia are designated influenza. But it is equally certain that sporadic cases of the type of the epidemic of 1918 are occurring; in those developing pneumonia the mortality is high.

#### BRONCHIAL ASTHMA

This case occurred in a man of fifty-two years who had had attacks since early childhood. His paternal grandmother and father had the disease and one of his children now has it—a family history of four generations. Curschmann's spirals were found in the bronchi. The lungs showed emphysema. The bronchi had an acute catarrhal inflammation. Twelve years ago while in Berlin I had the opportunity of studying and reporting the eighth case in medical literature with microscopic examination of the lungs. This I believe to be the twelfth. These lungs shed no new light on the pathology of the disease, although the duration of the case should have led to chronic changes if any ever occur.

#### TUMORS

There were 9 cases of tumor, 7 malignant and 2 benign. Of the former, 4 were carcinoma, 2 sarcoma, and 1 hypernephroma. One case of carcinoma of the stomach was in a woman of forty-two. It was of the diffuse type with thickening and ulceration of the entire wall. It extended to the surrounding tissues but gave no metastases to the liver. A squamous-cell epithelioma of the uterine cervix in a woman of sixty-seven had extended to the bladder. A third case in which carcinoma had caused death was in a man of thirty-four. The liver was the organ chiefly affected, being studded by nodules of various sizes. The duodenum was adherent to one of the nodules and when separated was found to have a small perforation, at the base of an ulcer. The case was taken to be one of primary carcinoma of the liver, with multiple nodules from early spread by the veins. Microscopic study of the duodenal lesion showed a chronic ulcer with very thick walls of dense fibrous tissue and in this at one point a few aberrant epithelial cells that may be part of a malignant growth. These cells are too few to make the diagnosis of cancer positive but makes it strongly probable. The case is therefore better regarded as one of chronic ulcer of the duodenum with carcinomatous change and multiple metastases in the liver.

An instance of undoubted carcinomatous change in a chronic ulcer was found in a man of sixty-two years. In the greater curvature of the stomach was an ulcer 6 cm. in diameter, with a nodular elevation in the center that proved to be adenocarcinoma. There were no metastases, death being due to gangrenous cholecystitis.

Of the sarcomas, one was a lymphosarcoma of the duodenum in a woman of thirty-six years, with symptoms of six months' duration. A perforation 3 cm. in diameter had occurred; this was adherent to the gall bladder but leakage had started peritonitis. The second, third, and fourth parts of the duodenum were

an ulcerated, thickened mass of partly necrotic tissue with narrow segments of the lumen. Microscopically the growth was of small round cells and could have been called a small round-cell sarcoma but was so similar to the lymphnodes it infiltrated that the term lymphosarcoma is regarded more appropriate. This belongs to the rarer tumors in this location.

The other sarcoma was also one of infrequent occurrence, a primary sarcoma of the liver. This was in a Chinese woman of thirty-one, with an obscure history as to duration but probably about six months. A large mass with almost parallel sides and a rounded end extended from the lower part of the right lobe of the liver diagonally across the abdomen to a point below and to the left of the umbilicus. A needle puncture gave pure blood. Opening the abdomen revealed an inoperable mass of soft reddish tissue that under great pressure welled out of the incised capsule. Postmortem showed a growth of the liver replacing more than half of the right lobe and extending 15 cm. beyond the border as a cylindrical mass 12 cm. in diameter. The tumor was extensively necrotic with many areas of hemorrhage. There was no tumor elsewhere in the body. Microscopic sections are of round-cell sarcoma.

The other malignant tumor was a hypernephroma 16 cm. in diameter that replaced the upper two-thirds of the right kidney and the adrenal. There were multiple secondary nodules in the liver and lungs.

Of the benign tumors, one was a hemangioma of the liver 3 by 4 cm. in a woman of fifty-four. The other was a lipoma 2 cm. in diameter projecting into the lumen of the middle of the ileum of a woman of 50 years.

#### TYPHOID FEVER

There were three cases of this disease in males of twenty-one, eighteen, and seven years. The first two mentioned had extensive lesions in the lower six feet of the ileum and both had numerous small ulcers in the cecum. The first had the most marked inflammation of the mucosa of the ileum that I have ever seen in a case. The boy had but little lymphoid tissue in the ileum, therefore but few lesions; there were none in the cecum and the ileum between the ulcers was not inflamed. But the ulcers present were deep and one 10 cm. above the ileocecal valve had perforated and caused peritonitis. Comparison of these cases emphasizes a point of great importance regarding the lesions of this disease, namely, that perforation does not necessarily imply an extensively inflamed or ulcerated intestine. Of these three cases, perforation occurred in the one with the least lymphoid tissue in the intestine, with the shortest length of intestine involved, with the fewest ulcers, and the least inflammatory tissue around the ulcers.

In addition to these three cases of typhoid fever, the ileum of a woman of fifty-one contained bile-stained scars that were probably the remains of lesions from which she had recovered. There have been several other cases of the disease in the hospital during the past year. Typhoid therefore appears to be an endemic disease in Bangkok although some physicians were skeptical about it when I first came. There are so many types of fever in the tropics, many of them of unknown origin, that proof of specific diseases is demanded.

## MULTIPLE ANEURISMS OF HEART

These were in a woman of unknown age and formed an instructive instance of the effect of sclerosis of the arteries. One aneurism was a cone-shaped projection 2.5 by 1 cm. at the apex of the left ventricle. At the summit the wall of the sac was formed of pericardium only, but the terminal 1.5 cm. was occupied by a blood clot partly organized into fibrous tissue. This, as fortunately often happens in such cases, undoubtedly prevented rupture of the aneurism. The second aneurism had an opening 2 cm. in diameter leading from a point behind the posterior mitral leaflet. The sac was 4 by 2 cm. in size and was partly filled by partially clotted blood. This sac was also thin. Both were apparently due to local failure of blood supply from occlusion of small branches of the coronary artery. Other effects of arteriosclerosis in this subject were fibrous myocarditis and softening of the brain.

## JAUNDICE IN ATROPHIC CIRRHOSIS OF LIVER

Two of the subjects with atrophic cirrhosis of the liver had pronounced jaundice. This condition is rarely marked in so-called atrophic cirrhosis and even in lesser degrees is present in only a small number of cases. Its occurrence in one case was unexplained by any condition that could be found. The subject was a man who had 9 liters of fluid in the peritoneal cavity. The liver was of the "hobnail" variety. The ducts were open. The liver was greenish-yellow.

The second case was in a man with "hobnail" liver and ascites of 10 liters. The jaundice in this case was readily explained. Dense adhesions of the mesocolon to the pedicle of the liver and along the duodenum almost to the pylorus had added obstruction of the bile ducts. The jaundice was thus really obstructive. Edema of the colon in this man was the most intense I have seen. In some segments the wall was 1.2 cm. thick.

## LOBAR PNEUMONIA

This affection is very uncommon as compared to the United States during the time of year embraced in these statistics. Although the "cold" season here (it was not below 55 degrees the past winter) causes many mild or even more severe cases of inflammation of the upper respiratory tract, judging from this series it does not lead to pneumonia in any notable number of cases. The one case here noted was in a man of forty who had a number of chronic diseases with the pneumonia as somewhat of a terminal event. It was of the type known as "central" pneumonia.

## INTESTINAL PARASITES

The cases of these were as follows: Hookworm, 1; Tapeworm, 1; *Ascaris lumbricoides*, 2; *Entameba histolytica*, 17.

The hookworms were found in a boy of sixteen, who had marked anemia of all his organs. One ascaris was found. Examination of this patient's stools

when admitted to the hospital showed the presence in his intestines of hookworms, ascaris, strongyloides, and trichuris.

The tapeworm was found in the intestine of one of the subjects of typhoid fever. It was the saginata in type.

The ascaris was found, in addition to the case mentioned, in a man of sixty-two, previously described as the patient having carcinomatous change in an ulcer of the stomach and suppurative cholecystitis. Two worms were found in the stomach and four in the common bile duct and liver. The latter had apparently wandered into the liver postmortem, as there was no evidence of lesion in the organ. Infestation by this parasite is world-wide, but it is especially frequent in the tropics. Ova are found in the feces of many of the patients admitted to the hospital here.

#### AMEBIC COLITIS

The fact that 17 cases of amebic dysentery occurred in this series of 100 is sufficient proof of the prevalence of that disease. Of these cases, 10 were men and 7 women. The lesions in the colon varied from superficial necrosis without ulceration to the presence of many large, well-defined ulcers or to deep necrosis of the entire mucous membrane of the colon. Perforation occurred in two cases but adhesions prevented infection of the peritoneum in one; the other terminated in death from peritonitis. It would be tedious to describe all these cases but three may be briefly cited as illustrations.

One of the striking acute cases occurred in a man of fifty-four. He was ill 12 days, having 15 or more stools daily. Emetin hypodermically had no effect. At postmortem the colon was found involved the entire length. There were numerous ulcers from 2 to 8 cm. long. Some had a clean base of red tissue, others were partly covered by necrotic tissue. In some segments the ulcers were very closely placed, in others separated by broad patches of gray, swollen necrotic tissue. Apparently the entire mucosa of the colon had either been lost by ulcer formation or had become necrotic and was still attached. The ulcers as a rule were not excessively deep but 20 cm. from the head of the cecum was one 4 cm. in diameter that had a base of yellow or brown dead tissue, with the peritoneal surface showing the same color. In this area were three small perforations; around them were adhesions but leakage had occurred and peritonitis developed. Microscopic sections of deep ulcers show necrosis of the submucosa or even into the muscle coat. In the deeper necrotic tissue and along its junction with the living are great numbers of amebae. This case is an example of extensive ulceration.

Another type of involvement was found in a woman of 28. The wall of the cecum was thickened and the whole of its mucosa yellow or greenish in color but still attached and quite firm. This condition became gradually less marked further down in the colon. At points were a few shallow ulcers, but ulceration was not at all a feature. Microscopically necrosis was very superficial and amebae were not deep in the wall. The submucosa was swollen and contained fibrin. The process also extended 15 cm. into the ileum, involving the mucosa uniformly as in the cecum, and ending abruptly in a perfectly straight line

entirely around the intestine. This was an instance of superficial necrosis of the intestine of the so-called diphtheritic or gangrenous type with very little ulcer formation.

A third type occurred in a man of forty-four years; duration of disease one month. All the mucosa of the colon and in many areas the submucosa also was necrotic and hanging to the wall as soft grayish-yellow shreds. There were no definite ulcers but at points, especially in the sigmoid, the dead tissue was easily detached, leaving a very thin wall. Along the sigmoid the omentum was adherent; there was no actual perforation but this was evidently imminent. At many places in the deeper layer of dead tissue were small areas 0.5 to 1 cm. in diameter of yellow, semifluid, tenacious substance resembling thick pus. In the tip of the cecum the necrosis was more shallow and more like the diphtheritic type. This same condition but to a less marked degree was present in the lower 30 cm. of the ileum. Above this a 15 cm. segment was hyperemic, then necrosis for 15 cm., thus making the lower 60 cm. of the ileum affected.

These three cases may be taken as types or illustrations. The first was one with many ulcers of various sizes, most of them not deep, but one perforating. The other two did not have many ulcers. The one had moderate destruction of tissue, the other extensive necrosis. The remaining 14 cases showed various degrees of involvement of the colon, most of them having ulcer formation.

It will be noted that in two of the three cases cited the ileum was involved. A third case with lesions in the ileum was in a woman of fifty-six with numerous ulcers and extensive necrosis of the colon. The lower 20 cm. of the ileum was affected in the same way except ulcer formation was not so extensive. This makes three of the 17 cases with lesions of the ileum, a larger proportion than the literature would lead one to believe occurs. Of course such a short series cannot be taken as establishing a percentage of great value.

#### AMEBIC ABSCESS OF THE LIVER

Three of the cases of amebic dysentery were complicated by abscess of the liver. One was chronic, the others were in the acute stage.

The chronic abscess was in a man of forty. The hepatic flexure of the colon, the duodenum, and the right kidney were adherent to the liver. Through these adhesions the abscess had opened into the duodenum by an opening 2 by 1.5 cm. More than half of the right lobe of the liver was occupied by a cavity containing thick reddish fluid. The wall was fairly smooth, of necrotic liver with a thick layer of new fibrous tissue beneath. In scrapings from the wall were motile amebæ. The lesions in the colon were not extensive. A segment of the lower sigmoid was studded with small ulcers a few millimeters in diameter. The remainder of the colon had only areas of congestion.

One of the acute abscess cases was in a man of 51 years. The conditions present were misleading and the appearance of the lesions in the liver were suggestive of pyogenic rather than amebic origin. The man had gangrenous appendicitis with periappendiceal abscess and peritonitis. In the liver were three cavities, 2 in the right, one in the left lobe, from 2.5 to 4 cm. in diameter and filled with thick yellow fluid, resembling pus. The walls were quite thick

and somewhat rough, the only point against the supposition that the abscesses were metastatic from the appendix. Microscopically the abscesses were typically amebic, including many amebae. The colon had extensive ulcerative lesions in which were small numbers of amebae.

The third case of amebic abscess of the liver was in a woman of thirty-three years. She had shortly before been delivered of a dead fetus, had repeated postpartum hemorrhages, and exhibited hemorrhagic endometritis. The liver had fatty infiltration. Throughout the organ, but especially in the right lobe, were numerous cavities from 0.2 to 1.5 cm. in diameter and filled with thick, tenacious, pale yellow fluid. The walls were rough by necrotic tissue. The colon was the site of numerous ulcers in its whole length. Many of these were at the summits of elevated areas the basal portion of which was occupied by thick yellow fluid like that in the liver abscesses. Sections of colon and liver abscesses were those of amebic lesions.

This suppurative appearance of early amebic abscesses of the liver in the second and third cases as opposed to the large abscess in the first with its characteristic chocolate-colored fluid, was very striking. Clinical experience would indicate that when abscesses are large enough to give symptoms and thus become of importance from a diagnostic standpoint, they contain fluid that makes diagnosis practically certain. The different appearance of the small ones therefore would seem to be of pathologic rather than of clinical interest.

Many other findings in these postmortems were of very great interest but their recital would make tedious what has been intended to serve as a brief indication of the material with which we are working. A vast field in investigative tropical medicine is here waiting to be tilled.



## A CASE OF SPONTANEOUS, ACUTE AND SUBACUTE PEPTIC ULCERS AND CARCINOMA OF THE THYROID IN A DOG\*

By F. C. MANN, M.D., ROCHESTER, MINN.

A SPONTANEOUS gastric or duodenal ulcer is so rare that it may be of value to report finding two gastric ulcers of different degrees of chronicity and an acute duodenal ulcer in a dog with carcinoma of the thyroid. The older gastric ulcer did not show signs of as great chronicity as the peptic ulcer in man, but it more nearly approached such a lesion than any ulcer which I have seen in an animal.

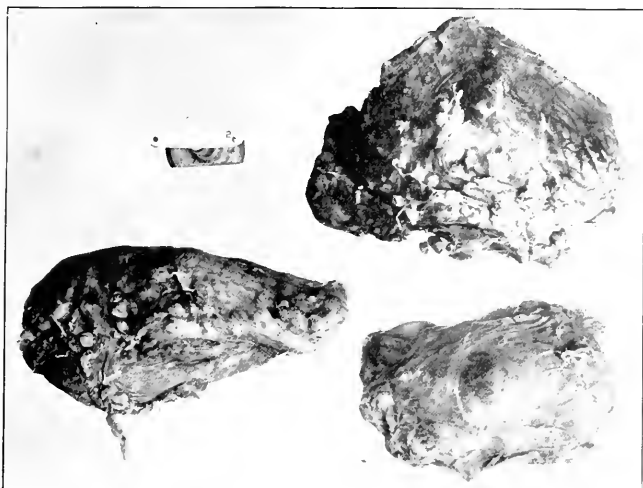


Fig. 1.—Gross specimens of the thyroid and substernal growth. Relative position and size.

### DETAILED PROTOCOL

Dog D 519. An old male, Boston bull-terrier, weighing 12.5 kg., was brought to the laboratory Feb. 27, 1920. The animal was very thirsty and drank a large quantity of water which was vomited up almost immediately. At first this excessive drinking was considered responsible for the vomiting. The vomiting continued, however, and the animal ate very little; it died during the morning of February 28.

Necropsy 92 was performed shortly after death. No postmortem changes had occurred. The animal was in good general condition; the omentum was

\*From the Division of Experimental Surgery and Pathology, The Mayo Foundation (Graduate School, University of Minnesota).

filled with fat and there was a thin layer of subcutaneous fat. The animal was very old; the teeth were worn down almost to the gums and many were missing. However, no alveolar abscesses were noted. Tonsils could not be found. The thyroid glands were markedly enlarged and nodular. On removal they appeared to show malignant growth throughout. The centers were degenerating and contained many small hemorrhagic areas. The isthmus of the thyroid seemed to be present and projecting from it down into the upper anterior mediastinum was a large growth equal in size to the lobes of the thyroid. Measurements of these lobes were as follows: the right thyroid was 10 cm. by 6 cm.; the left 7 cm. by 4 cm. and the substernal portion was 9 cm. by 5 cm. The three glands ap-

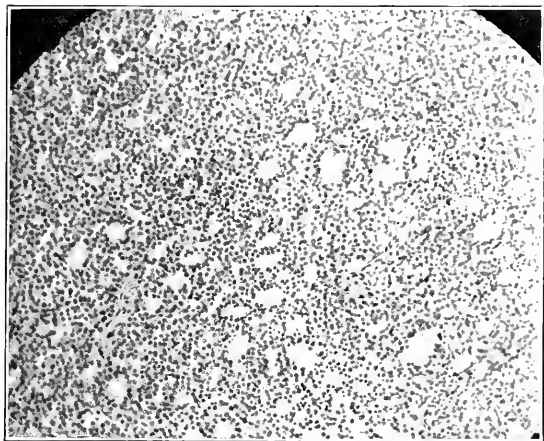


Fig. 2—Photomicrograph of an area of the substernal growth. Note the thyroid-like arrangement of some of the carcinoma cells. Magnification  $\times 100$ .

peared alike grossly, both intact and on section. They were nodular, the surface quite vascular, and on section they showed large granular nodules interspersed with many large and small hemorrhagic areas (Figs. 1 and 2).

Each lobe of the lung contained from three to eight hard, almost spherical nodules, measuring from 4 mm. to 3 cm. in diameter. On section they were quite homogeneous and faintly granular. They appeared undoubtedly to be metastasis (Figs. 3 and 4).

The abdominal cavity seemed to be free from any infection and at first no pathologic condition could be found except a few small hemorrhagic areas throughout the pancreas.

The kidneys showed a slight amount of nephritis. There were a few small adenomas in the right adrenal. Grossly the spleen, liver, bladder, and prostate appeared to be normal.

When the stomach was opened a large indurated ulcer with raised edges was found 2.5 cm. from the pylorus. This ulcer measured 8 mm. by 15 mm. and

extended through to the muscularis. About 1 cm. from this ulcer, and an equal distance from the pylorus, was another ulcer about 4 mm. in diameter. This ulcer was more acute than the larger ulcer, its walls not being nearly so much indurated. Another ulcer, about 12 mm. in diameter was found just beyond

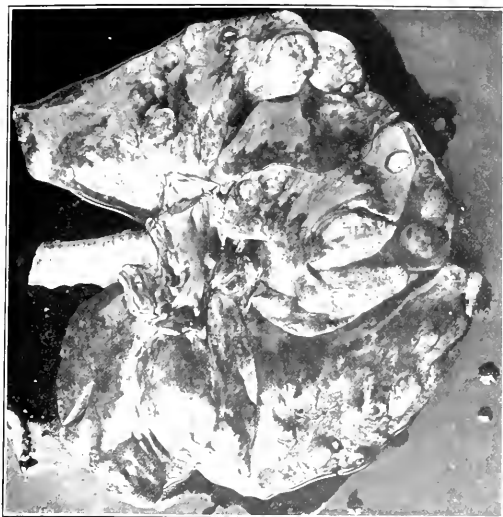


Fig. 3.—The lung showing a large number of metastatic areas.



Fig. 4.—Photomicrograph of section of one of the metastatic areas of the lung. Compare with Fig. 3. Magnification  $\times 100$ .

the pyloric ring, in the duodenal mucosa; it had perforated the entire duodenal wall, but was sealed with a thin layer of gastrohepatic omentum; below this was an edge of the pancreas and the edge of a lobe of the liver. The seal was broken while the stomach was being pulled up gently, showing the ulcer perforated into the lesser peritoneal cavity, but no infection was noted (Figs. 5, 6, and 7). The liver showed no evidence of metastasis, and, with the exception of a submaxillary lymph node, no other evidence of malignancy was found elsewhere in the body.

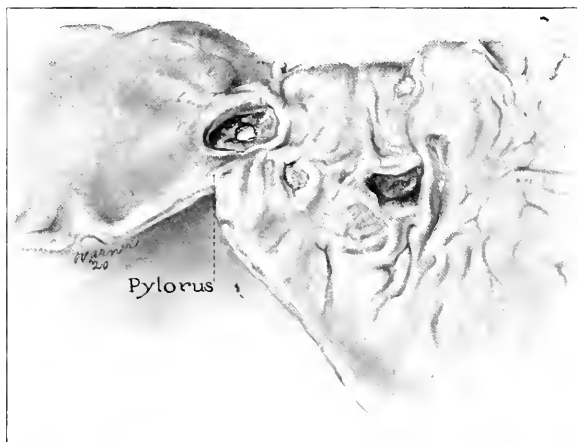


Fig. 5.—Drawing of a location and comparative size of the ulcers. The larger ulcer of the gastric mucosa has a hard indurated base and overhanging edges. The ulcer in the duodenum has completely perforated the duodenal wall.

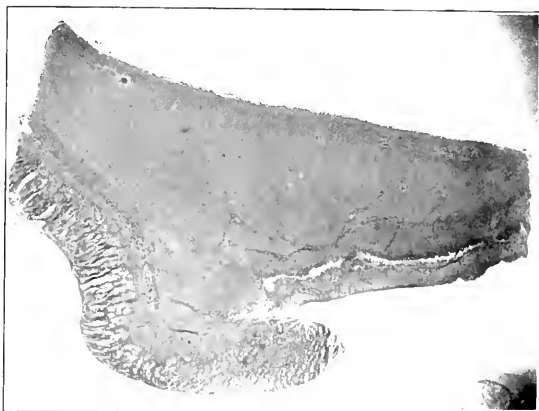


Fig. 6.—Photomicrograph of one edge of the larger gastric ulcer. Note the overhanging edge of the ulcer and the layer of necrotic cell debris at the base. Magnification 89.

## MICROSCOPIC EXAMINATION

Microscopic examination of sections of each of the three thyroid specimens showed carcinomatous changes. The metastasis in the lungs also was rather characteristic of a carcinoma of the thyroid. Histologic sections of the ulcers showed the picture characteristic of such conditions. The ulceration in the acute duodenal ulcer was clean-cut, the walls appearing almost as though cut by a knife. There was but slight reaction around the edge of the ulcer which penetrated all coats of the duodenum. The appearance of the larger gastric ulcer was more suggestive of chronicity. It had an overhanging edge of mucosa and the base was covered with necrotic cell debris. Hemorrhagic areas were



Fig. 7.—Photomicrograph of the base of the ulcer shown in Fig. 6. Note the proliferation of young connective tissue. Magnification  $\times 100$ .

found in the muscularis at the base. The connective tissue around the edge and at the base had begun to proliferate and had undoubtedly produced the induration which could be felt on palpation.

The development of the ulcers, it might be reasoned, followed embolic plugging by carcinoma cells of the blood vessels supplying the areas involved. However, there was no evidence of malignant cells in the stomach, and the fact that metastasis could be found nowhere but in the lungs mitigates against such a theory.

# LABORATORY METHODS

## A SIMPLE METHOD FOR THE REMOVAL OF NATURAL AMBOCEPTOR FROM HUMAN SERA\*

BY R. L. KAHN, SC.D., LANSING, MICH.

### INTRODUCTION

JUDGING from recent discussions on the removal of natural amboceptor from human sera, it appears that the problem involved is not whether the removal of amboceptor is essential for correct Wassermann tests—this it seems is quite established—but the fact that there apparently exists no simple method for removing amboceptor which meets the requirements of laboratories performing large numbers of tests daily.

The work of Ottenberg,<sup>1</sup> Simon,<sup>2</sup> and others, and more recently that of Kolmer and Rule,<sup>3</sup> definitely proves the importance of removing natural amboceptor from the patients' sera in the Wassermann test. Olmstead's<sup>4</sup> contention that if the tests are read as soon as the controls show complete hemolysis, instead of after keeping them in the ice box overnight, that the presence of natural amboceptor is likely to be insignificant, is in part corroborated by Kolmer and Rule. These investigators report only a very small per cent of false negatives due to natural amboceptor when reading the tests as soon as the controls haemolyze. Granting that the factor of error is not more than one per cent, it is evident that no laboratory can afford to permit this error to enter in its daily work.

The problem involved, however, as was indicated above, appears to be the fact that there is no method for amboceptor removal which is sufficiently practical to render it widely applicable in routine Wassermann laboratories. To quote Von Wedel,<sup>5</sup> "all methods of removing the natural antisheep amboceptor are probably too time-consuming for a routine diagnostic laboratory to perform when large numbers of sera are examined each day." Kolmer and Rule also, in discussing this phase of the question, state that "All methods for the removal of antisheep hemolysin are too time-consuming and laborious for routine absorption of all sera \* \* \* when the examination of fifty or more sera constitutes an ordinary day's work."

The proposed method for the removal of natural amboceptor from the patients' sera, has been applied to 10,000 Wassermann tests and in our opinion meets the requirements of laboratories performing large numbers of tests daily. In this laboratory with about 70 to 130 daily examinations, the element of delay produced by our amboceptor removal method, is practically negligible. Although some theoretical phases of our procedure are still under investigation, we nevertheless feel, in view of the above quotations, that it would be well to present our method at this time.

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## THE METHOD

The method is based on the well-known affinity of sheep-cells for antishcep amboceptor, and consists of adding packed sheep-cells to inactivated serum in the proportion of one drop per c.c. of serum, and permitting the extraction to take place for 10 minutes at room temperature.

In applying this simple procedure, the following factors are to be considered. The packed sheep-cells employed are part of the same cells which, after proper dilution, are used in making the sheep-cell suspension for the Wassermann tests. A quantity of sheep-cells are washed daily with a view of having several extra c.c. of packed cells for the absorption of amboceptor. The drop employed is somewhat smaller than ordinary size, comprising 25 to 30 drops per c.c.

As soon as the serum-tubes are taken out of the inactivating bath, they are lined up in such a manner that variations in quantities of the sera may be easily observable. A drop of packed sheep-cells is then placed in each tube containing approximately one c.c. and shaken gently. To those tubes containing less than one c.c., a part of a drop is permitted to touch the inner side of the tube and the serum brought in contact with the cells by slightly slanting the tube.

Just as soon as cells have been added to all the sera, the tubes are immediately placed in centrifuge holders; balanced, and placed in the centrifuge. By this time, the ten minute extraction period is usually completed; the centrifuge is then started and permitted to run from 6 to 8 minutes. During this centrifugation period, a series of clean tubes are numbered corresponding to those which are in the centrifuge. After centrifugation, the clear supernatant sera are poured into the newly numbered tubes and are ready for use in the Wassermann tests.

## DISCUSSION

For an amboceptor removal method to have wide acceptance, it must first be of proved efficacy; second, it must not render the sera anticomplementary and third, it must not be unduly time-consuming.

1. The efficacy of this simple procedure is based on studies carried out in this laboratory on the rate of absorption of amboceptor by packed sheep-cells at various temperatures. A complete report of these studies will be presented in another paper. Suffice it to say in this connection, that it was observed that if an ordinary sized drop of packed sheep-cells is added to 1 c.c. of serum containing 200 units of amboceptor that this small quantity of blood will absorb as many as 160 units of amboceptor in 5 minutes at room temperature. The employment, therefore, of a ten minute absorption period in our procedure, gives a sufficient margin of safety for the absorption of far more amboceptor than is likely to be present in human serum.

We have further tested the dependability of our simple method in the following manner: After fixation of complement by serum and antigen in our regular Wassermann tests, sheep-cells were added (without amboceptor) and incubated in the water-bath at 37° C. for 10 minutes (Kaliski procedure). Natural amboceptor, if present, would thus have had ample time to produce hemolysis of the cells. We have not, however, observed a single instance of

hemolysis—not even in the smallest degree—in 300 such tests carried out at different times, thus proving the efficacy of this procedure.

It might be added that in our opinion, the slight dilution produced by a drop of packed cells in 1 c.c. of serum, does not affect the accuracy of the test. The cells are packed down by centrifugation for 14 minutes at 1600 r. p. m. and if the drop to begin with, is about  $\frac{1}{25}$  of a c.c. the amount of saline which remains with the serum, may safely be considered negligible.

2. It is well known that sera acquire anticomplementary properties after prolonged extraction with red cells at incubator temperature, and in order to overcome this property, Rossi<sup>6</sup> suggested a procedure for amboceptor absorption at low temperature. This worker employed chilled centrifuge tubes, chilled corpuscles, and centrifuged in the cold (during warm weather) after keeping the extraction mixture in the ice chest for 30 minutes. The difficulty of applying this method on a comparatively large scale, is very evident.

In our studies on the rate of absorption of amboceptor by sheep-cells, referred to above, we have observed the occasional development of slight anti-complementary properties after a thirty minute, but not after a ten minute extraction period. This anticomplementary phase of our procedure is still under investigation, but there is every indication that a room temperature extraction lasting only 10 minutes, is not sufficient for the development of anticomplementary properties.

3. Regarding the time-consuming element of our procedure, we find that it takes less than five minutes to add 100 drops of sheep-cells to the same number of serum tubes; 10 minutes to balance the tubes for centrifugation; 10 minutes for centrifugation, and 10 minutes to pour the supernatant clear sera in other tubes. These steps are carried out by one worker, while another is looking after the dilution and titration of complement. It is thus evident that the delay produced by this absorption procedure in the completion of the daily tests, is quite insignificant.

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- <sup>5</sup>Von Wedel: Jour. of Immunology, 1920, v, 159.
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## LACTOSE—DETERMINATION OF IN MILK BY COLORIMETRIC METHOD\*

BY R. G. OWEN, M.D., AND ROTH GREGG, DETROIT, MICH.

FOLIN AND McELLROY<sup>1</sup> have described a titration and a colorimetric method for the determination of lactose in cow's and human milk, both of which give quite accurate results. In a later paper, Folin and Peck<sup>2</sup> give more

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elaborate directions for the preparation of the reagents used in the titration method. Pacini and Russell<sup>3</sup> have likewise described a colorimetric determination of lactose. All these authors make use of picric acid as a protein precipitant.

In their system of blood analysis Folin and Wu<sup>4</sup> have developed the use of tungstic acid as a protein precipitant, and as the use of their reagents and methods have come into quite universal use it occurred to us that the use of these same reagents and technic might simplify the determination of lactose in samples of breast milk.

Our method is simply an adaptation of Folin's<sup>5</sup> latest blood sugar determination to the estimation of lactose. The reagents used are those described by Folin.

#### METHOD

To 1 c.c. of milk in a 100 c.c. flask add 2 c.c. of 10 per cent sodium tungstate and 2 c.c. of  $\frac{2}{3}$  normal sulphuric acid, the latter drop by drop. Mix and let stand 5 minutes. Dilute to 100 c.c. and filter. We find that 2 c.c. each of tungstate and acid give clearer filtrates than where only 1 c.c. of each is used.

Place in a Folin special sugar tube 1 c.c. of filtrate and 1 c.c. of water, to balance volume in tube, add 2 c.c. respectively of standards A & B to two other sugar tubes. To the unknown and the standards add 2 c.c. of alkaline copper solution and place in boiling water 6 minutes. Place tubes in cold water until cooled, then add 2 c.c. of Folin molybdate phosphate solution and let stand a few minutes.

Dilute the three solutions to 25 c.c., mix and read in the colorimeter, using that standard which most nearly matches the unknown and setting the standard at 20.

Standards. Make up an accurate 1 per cent lactose solution in distilled water using purified lactose. We have obtained our lactose from the Digestive Ferments Co., Detroit, Mich. Add a little toluol. We have found that this stock solution keeps well for several months. Standard 1, of which 2 c.c. contains 0.5 mg. lactose is made by diluting 12.5 c.c. of the 1 per cent stock lactose solution to 500 c.c. Standard 2, containing 0.7 mg. per 2 c.c. is made by diluting 17.5 c.c. of the 1 per cent solution to 500 c.c. Preserve these solutions with toluol and make up fresh about once a month.

$$\frac{\text{Standard Reading}}{\text{Unknown Reading}} \times 5, \text{ if standard 1 is used, or times 7 unknown reading}$$
if Standard 2 is the one selected will give the grams of lactose per 100 c.c. of milk.

Figures obtained by the above method of estimating lactose check very well with the Folin titration method. Moreover, lactose added to various samples of milk can be quantitatively recovered to within a few milligrams.

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- <sup>3</sup>Pacini and Russell: Ibid., 1918, xxxiv, 505.
- <sup>4</sup>Folin and Wu: Ibid., 1919, xxxviii, 81.
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## A SIMPLE SHAKING DEVICE

By J. P. BAUMBERGER, STANFORD UNIVERSITY, CALIFORNIA

RECENTLY we had occasion to make an impromptu shaking device which has proved very satisfactory. The apparatus makes use of a discarded foot-driven machine similar in structure to a sewing machine. As much foot-driven apparatus is available in laboratories, or can be readily obtained for a song, we believe the following description will not be amiss:

An old color-mixing bench formed the principal part of the apparatus. The driving rod (*E*, Fig. 1), of the bench, extending from the foot-pedal to the crank-shaft (*D*) was disconnected from the pedal and fastened with strap iron (*F*) to the middle of the upper edges of the end and partitions of a box (*G*).

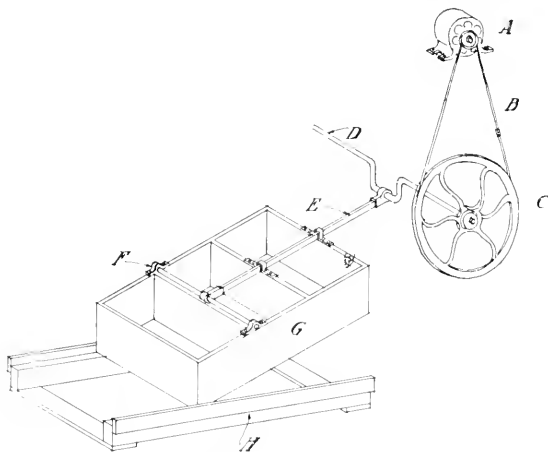


Fig. 1.—A new shaking device. (See description in text.)

The driving rod is prevented from turning by an iron rod at one end as shown at (*F*). The driving rod thus served as an arm suspending the box from the crank shaft, while the other end of the box rested on two greased runners (*H*) at a convenient height. It should be noted that the driving rod is fixed firmly to the box and not with a flexible joint as in some devices on the market, and this (former) system makes a more effective shaker. The grooved drive wheel (*C*) of the machine was then belted (*B*) to the pulley of a motor (*A*) and the apparatus was complete. Upon turning the drive wheel the crank-shaft gives the box a forward, upward, backward, and downward motion which is very effective in shaking the contents of flasks, etc., which may be packed in excelsior in the box. The section of the box nearest the crank-shaft is most vigorously shaken and the third section only gently agitated.

## DETERMINATION OF COAGULABLE PROTEIN IN SERUM\*

By W. N. BERG, PH.D., WASHINGTON, D. C.

**T**HEORETICALLY, the determination of total coagulable protein in serum or a similar material is simple. It is only necessary to dilute the serum with about 20 volumes of water, add dilute acid to neutrality, heat to the boiling point and the coagulable protein is precipitated in large flocculi which makes subsequent filtration easy. The precipitate is weighed after drying to "constant" weight. In actual practice the numerous sources of error lead to the use of indirect methods; for example, the protein content of a serum is determined, by the use of the refractometer; the nephelometer, etc. For a discussion of these methods see Robertson.<sup>1</sup> The method of filtering the coagulated protein on to weighed filter papers, and weighing the dried precipitate plus the paper in weighing bottles, as described by Berg<sup>2</sup> is open to two objections as previously pointed out: (1) the difficulty of drying filter paper and coagulated protein to constant weight in such fashion that while water is being driven off, oxidation shall not take place; (2) the method requires comparatively large quantities of serum, i.e., 5 or 10 c.c. This is more than is available in many immunologic studies.

A method was desired that conformed to the following requirements: (1) it must be direct, so far as the coagulable protein is actually coagulated, dried and weighed; (2) a single determination must be possible in a small quantity of serum, i.e., 0.50 c.c.; (3) the coagulable protein obtained and weighed must represent the actual total coagulable protein in the original sample with a high degree of accuracy. The method herein described meets these requirements. Considering the results obtained, its advantages over other methods justifies its use.

### PIPETS

One c.c. pipets graduated into 0.01 c.c., were used for measuring the 0.50 c.c. portions of serum used in a single determination. The length of these pipets between the 0 and 1.00 c.c. marks was approximately 250 mm., so that reading the pipet to a small fraction of 0.01 c.c., was not difficult. These pipets were specially made; because the usually graduated pipets have the 0.01 c.c. divisions too close together. An error in measurement of 0.01 c.c. of serum is unnecessary with properly made pipets. All were standardized by the Bureau of Standards.

The question naturally arises whether a pipet of the Mohr type, which will deliver 1.00 c.c. of water will deliver 1.00 c.c. of serum which is much more viscous than water. The following results were obtained from three concordant weighings of water and serum delivered by a 1.00 and 5.00 c.c. pipet; the sample of Berkefeld filtered normal horse serum had a specific gravity of 1.0354

\*From the Pathological Division, Bureau of Animal Industry, United States Department of Agriculture.

at 30° C., and contained 0.0752 grams solids-not-ash in 1.00 c.e. One c.e. pipet No. 19814 delivers 0.994 c.e. water at 20° C., according to Bureau of Standards certificate. This pipet delivered 0.990 c.e. water or 0.979 c.e. serum at 32° C. Similarly, 5 c.e. pipet No. 19820, certified to deliver 5.010 c.e. water at 20° C., delivered 4.985 c.e. serum at 30° C. The observed difference between the volume of water and serum delivered in the 1.00 c.e. pipet, i.e., 0.994-0.979 or 0.015 c.e., was for unrestricted outflow; because the pipets are calibrated that way. But in actual measurement of serum for analysis, the outflow is restricted somewhat, so that delivery is slow; this makes for accurate reading of the pipet and diminishes the error due to viscosity. For all practical purposes the volume of serum delivered for a single determination, i.e., 0.50 c.e., differs from 0.50 c.e. by a negligible amount. The weighings were made in glass-stoppered, weighing bottles and corrections were made for loss by evaporation: this amounted to from 2 mgs. in 30 seconds to 6 mgs. in 3 minutes.

#### CENTRIFUGE TUBES

These were heavy-glass, bacteriologic test tubes without lip; outer dimensions, length 95 mm., diameter, 17 mm., average weight 11.5 grams; maximum capacity 13-15 c.e.; numbered consecutively with hydrofluoric acid. Before use these were cleaned in hot sulphuric-acid, potassium-bichromate mixture, rinsed in distilled water, dried at 100° C. and kept in a dessicator till needed. The same tubes can be used repeatedly; between determinations their weights did not vary more than 0.1 or 0.2 mg. Some of the tubes lost no weight after ten treatments with acid-bichromate mixture. Such tubes are much more constant in weight than dry filter papers; they are quickly weighed to a 0.1 mg. and are not hygroscopic. As an example, Tube No. 10, weighed originally 12.4946 grams, did not weigh less than 12.4944 or more than 12.4948 grams during the course of 11 weighings and 10 treatments with acid bichromate mixture in three years. None showed greater variation. Twenty-four is a convenient number to have on hand.

#### CENTRIFUGE

This was a medium-sized, motor-driven centrifuge, head approximately 180 mm. in diameter, carrying 8 tubes. It was run at 2400 revolutions per minute for 20 to 25 minutes during a determination.

#### COAGULATING THE PROTEIN

The samples of serum, taken from the refrigerator are allowed to come to room temperature. Determinations should be made in triplicate. Into each of three clean, weighed tubes, pipet 0.50 c.e., of the sample. Obviously the greatest care should be used to avoid error in measurement. From a buret measure 9 c.e. distilled water into each tube.

The object of the following procedure is to gradually neutralize the alkalinity of the serum with dilute acetic acid, aided by heat, and to coagulate the protein by bringing to a boil after neutralization is effected. In this way hydrolysis of protein by acid or alkali is avoided.

From 0.7 to 1.0 c.c. n 50 acetic acid are usually required for maximal precipitation of protein in 0.50 c.c. serum. Heat the tube over a small Bunsen flame until the tube is hot to the hand, but the contents do not approach a boil. Add the n 50 acetic acid, at first trying 0.2-0.3 c.c. A precipitate should form which will dissolve on rotating the tube, giving the contents an opalescent appearance. Add 0.2-0.3 c.c. more acid; each addition of acid precipitates protein which will redissolve when the tube is rotated, so long as the amount of acid added is insufficient for complete precipitation. Heat the tube after each addition of acid, but do not approach a boil. As the amount of added acid is increased, the contents become more and more milky, until presently the protein flocculates. This can be easily observed. Add no more acid; heat the tube almost to a boil, let stand a few minutes. Time required for flocculating one tube is about 5 minutes.

## SOME TYPICAL ANALYSES

DESCRIPTION OF PRODUCT	USED IN EXP. NO.	DATE OF DETERMINA- TION OF COAGULABLE PROTEIN	MILLIGRAMS OF COAGUL- ABLE PROTEIN ACTUALLY WEIGHED FROM 0.50 C.C.		C.C. N/50 ACETIC ACID USED
Tetanus antitoxin 420 F	20	Aug. 8, 1917		31.2	0.1
				31.9	0.1
				32.1	0.1
	21	Oct. 12, 1917		32.5	0.1
				32.6	0.1
	3	Apr. 1, 1920		28.4	0.45
				31.9	0.55
	3	May 6, 1920		28.8	0.15
				30.0	0.15
				31.1	0.10
Tetanus plasma 9588	4	Apr. 20, 1920		28.4	1.1
				29.8	1.0
				36.5	1.1
	4	Apr. 23, 1920		33.0	1.2
				41.0	1.2
				42.9	1.2
	4	June 9, 1920		44.2	1.3
				44.8	1.3
				45.4	1.3
	Tetanus serum 2030	5	May 28, 1920		39.7
				39.8	0.9
				40.1	0.8
5		June 12, 1920		39.2	0.7
				40.5	0.8
				40.6	0.8
Diphtheria serum 2401	9	June 29, 1920		33.1	0.7
				33.2	0.7
				33.1	0.8
				35.1	0.8
				35.7	0.8
				35.3	0.9
				35.9	0.9
				36.0	0.9

When precipitation is maximal the supernatant fluid is water clear. If there is any cloudiness or opalescence, this is an indication that maximum precipitation has not been obtained. In such a case add 0.1 to 0.2 c.c. n 50 acetic acid, and heat for a minute. Sometimes this improves the result. Rotating the

tube so that the large flocculi mix with the fluid seems to improve matters by enabling the larger flocculi to pick up mechanically very small particles of coagulum. Obviously, the second and third tubes will generally give better results.

The tubes are centrifuged for 20 minutes at 2400 revolutions per minute. The coagulated protein packs firmly to the bottom of the tube. After centrifuging, the supernatant fluid is poured off: the tubes may be inverted without loss of coagulum. The reaction of the supernatant fluid in several determinations was found to be  $P_H = 6.6$ , showing that the precipitation took place in the presence of but traces of acid. Allow the tubes to drain well. The outsides of the tubes are cleaned by immersing them to about  $\frac{3}{4}$  of their height in strong sulphuric-acid, bichromate mixture, rinsing and drying with a towel.

The tubes are transferred to a vacuum desiccator and dried for 48 hours at room temperature; weighed, dried for a second 48 hours and again weighed to 0.1 milligram. The drying period may, however, be shortened. When the desiccator contains fresh acid, drying overnight generally gives a result that for practical purposes is correct. Further drying generally results in the loss of a fraction of a milligram. In every case constancy of weight was ascertained by at least two weighings.

The weights of coagulable protein in the subjoined table, typical of many others are the differences between the weights of the tubes and the tubes plus precipitate dried. When cleaned in bichromate mixture, washed, dried and weighed, the tubes are ready for the next determination.

Precipitates obtained from tubes containing a cloudy or milky supernatant fluid are invariably low and must be rejected. As the figures in the subjoined table show, the technic, properly used, will give results on the same sample that differ from one another by only a fraction of a milligram.

#### SOLIDS-NOT-ASH

It was considered desirable to ascertain what part of the total solids-not-ash in serum was precipitated by acetic acid in the determinations of coagulable protein. The solids-not-ash was determined as follows:

Duplicate portions of 1.00 c.c. of the serum were pipetted into small porcelain crucibles. These were transferred to a vacuum desiccator and dried at room temperature to constant weight. This gave the weight of total solids. The crucibles were then transferred to an electrically heated, muffle furnace and the contents ignited at a low red heat. Weight of total solids minus weight of ash gave solids-not-ash. These determinations can be carried out very accurately and involve no difficulty.

In nine series of determinations, on the products mentioned in the Table, the coagulable protein precipitated by acetic acid amounted to an average of 91.4 per cent of the solids-not-ash; the extremes were 89.2 and 94.2 per cent.

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- <sup>1</sup>Robertson, T. B.: *The Physical Chemistry of the Proteins*, New York, 1918, Chap. III.
- <sup>2</sup>Eichhorn, A.; Berg, W. N.; and Kelsor, R. A.: *Immunity Studies on Anthrax Serum*, Jour. Agric. Research, 1917, viii, p. 46.

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## EDITORIALS

### *Blood Sugar Tolerance in Cancer and in Hypertension*

THERE is no doubt that there are serious difficulties in diagnosis of gastrointestinal carcinoma, and that this is especially true of diagnosis in the early stages of malignancy. So, say Friedenwald and Grove, in spite of our modern methods of investigation, the diagnosis in many instances remains obscure often until late in the progress of the disease and is frequently not revealed until exploratory incision establishes the true nature of the affection. Any additional help therefore which may lead to the solution of this difficult problem cannot but be of the greatest value. In the blood sugar tolerance test these authors believe there has been added a procedure of real value.

The test is carried out in the following manner: After a night's fast 100 grams of dextrose dissolved in 300 c.c. of black coffee is given the patient. Just before this is given, blood is taken for a sugar test, and this procedure is repeated 45 minutes and 120 minutes after the ingestion of the sugar. The urine may be tested for sugar 120-180 minutes after the glucose meal, but is not essential.

The writers point out that Freund, in 1885, showed that there is always a hyperglycemia in carcinoma cases, and that there is none in sarcoma cases.

Later, Trinkler concluded that hyperglycemia is always present in carcinoma cases, and that in carcinoma of the internal organs this was of greater degree than in carcinoma of the skin or mucous membrane. He also pointed out that there was no relation between the hyperglycemia and cachexia. Rohdenburg, Bernhard and Krehbiel were the first to make careful investigations on sugar tolerance in cancer, and they showed that while a normal individual gives an increase in the blood sugar which reaches its maximum in 45 minutes and which then gradually falls to normal, the cancerous person, beginning with a normal blood sugar, shows a rise in 45 minutes, after which time it may continue to rise or remain stationary for 120 minutes after the glucose meal. At the end of this time the blood sugar begins gradually to recede and becomes normal not earlier than in 180 to 240 minutes after the feeding. Benedict and Lewis in a series of 53 patients showing malignancy, took the blood 180 minutes after ingestion of food. Of these 36 per cent showed a marked increase in blood sugar. At least 49 per cent showed a tendency to hyperglycemia. Benedict and Lewis point out that there is a steady increase in blood sugar as the disease progresses, and a maximum is reached just prior to death.

In the recent study of Rohdenburg, Bernhard and Krehbiel, based upon 228 cases, the authors call attention to three types of reaction following the sugar tolerance test. In the first the blood sugar at the 45 minute interval rises above the zero hour figure and at the 120 minute interval is as high or higher than at the 45 minute interval. In the second type the 45 minute reaction is similar to that of the first type, but at the 120 minute interval the figure is almost that of the zero hour. In the third type the initial blood sugar concentration is higher than, or the same as, that at 45 minutes, and at 120 minutes is the same or higher than the original figure. Nephritis in 75 per cent of the cases gave a type 2 reaction; tuberculosis in 60 per cent, gave a type 1; syphilis in 72 per cent, gave type 2; diabetes in 60 per cent gave a type 2; pregnancy in 66 per cent gave a type 1; 61 per cent of gastric carcinomas and 50 per cent of intestinal carcinomas gave a type 1 reaction.

Friedenwald and Grove studied 32 typical cases of carcinoma of the gastrointestinal tract. In addition they observed 3 cases of uterine carcinoma, 1 case of mammary cancer, 1 case of carcinoma of the prostate and spine, 1 case of uterine fibroid, 2 cases of sarcoma, 8 cases of peptic ulcer, 2 cases of gastric syphilis, 3 cases of diarrhea and dysentery, 8 cases of achylia gastrica and chronic gastritis, 4 cases of cholelithiasis, 2 cases of chronic appendicitis, 11 cases of enteropitosis, 5 cases of nervous dyspepsia, and 5 cases of intestinal stasis and mucous colitis. In addition they studied 4 perfectly healthy persons.

Their conclusions are that there is usually present in carcinoma of the gastrointestinal tract a rather characteristic curve of sugar tolerance which differs somewhat from that observed in carcinoma of other regions of the body. This curve presents a high sugar content, even in the fasting state, followed by an initial rise up to 0.24 per cent, or even higher within 45 minutes after the ingestion of the dextrose, remaining at this level for at least 120 minutes and at no time falling below 0.20 per cent. This test the authors say is rather distinctive and is of value in distinguishing between carcinoma and the other diseases of the digestive tract. As an aid in early diagnosis they are not definite,



but they say that since the reaction is characteristic regardless of the extent of the involvement it is to be presumed that it is present in early cases. They realize that the test is not specific of carcinoma and that it cannot be relied upon alone, in other words it is not pathognomonic. Nevertheless it appears that in cases of gastrointestinal disease in which there is some evidence of carcinoma, i.e., in differential diagnosis, the test may have a very important bearing, especially if diabetes, nephritis, tuberculosis, and thyroid disturbances have been excluded.

It has already been mentioned in the foregoing paragraphs that nephritis is one of the conditions in which glucose metabolism is upset, that nephritic patients react abnormally to the glucose tolerance test. With diabetes the same is true. Patients with chronic vascular hypertension may also show a disturbance in handling glucose. This type of case is very similar to the nephritic with hypertension. It is with a group of chronic vascular hypertension cases that O'Hare has concerned himself. He says that while following a large group of these cases it was noted that from time to time some of them showed a trace of sugar in the urine, occasionally enough to quantitate. The suggestions were made that these were cases of renal diabetes, or that they were incipient true diabetes. To test these suggestions the glucose tolerance test of Janney and Isaacs was used, i.e., the fasting blood sugar is determined and then 1.5 grams of glucose per kilogram of body weight, with 2.5 grams of water per gram of glucose was given. The blood sugar was determined 2 hours later, and the urinary sugar, 3 hours after the glucose meal.

The results of the application of this method are interesting indeed, as they appear in the chart in the original article, for, while certain cases by themselves give no striking results, when all are arranged in the order of the degree of the reaction, they form a scale which runs from normal to a point approaching the typical diabetic reaction. This suggests to O'Hare that these cases are potential diabetics.

—P. G. W.

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O'Hare: *ibid.* p. 366

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### *Present Day Immunology*

THE authoritative statement of present day immunology has been made; Jules Bordet has written a "Treatise on Immunity in the Infectious Diseases."<sup>1</sup> It will perhaps come as a surprise to those who have not followed attentively the investigations of the Belgian scientist to find that although great as a discoverer he is equally great as an expositor. Bordet is a master of that flexible and exact tongue that has no equal for scientific exposition and instruction. The French have a knack of repetition of the essential facts from multiple points of view with ingenious turns of phrasing that rejoices the ac-

<sup>1</sup>Bordet: *Traité de l'Immunité dans les maladies infectieuses*, Masson & Co., Paris, 1920.

customed eye or ear, and produces an indelible impression that is never equalled by the boiled down essence of protocols.

And the scientific use of the imagination that has made Bordet so fertile in experimentation is evident here also. The book is not simply an evaluation of acquired facts, but full as well of ingenious working hypotheses. The very success of this investigator lies in the fact that he has always approached new facts untrammelled by preconceived notions. And yet Bordet's explanation of his and others results, although strikingly free from convention, seems in last analysis the simplest explanation, the freest from unjustifiable assumption.

Bordet's book is an extensive one comprising over 700 pages and divided into four main sections dealing respectively with a general survey of the field; with cellular immunity; with humoral immunity; and with the reaction of the organism in its entirety. So far the treatise is orthodox enough. The well established and understood facts are fully and ably discussed. It is in the treatment of the ill-defined and undeveloped fields that the ingenuity of the author has full play and raises a series of ingenious questions and offers numerous suggestive hypotheses. It was some fifteen years ago that Bordet expressed to the reviewer the desire for a few months freedom from routine to indulge in an assembling and calm contemplation of the facts that had been demonstrated in his particular field. He felt the assurance that out of such a survey would come new leads of promise. The isolation came, not in the way he had imagined and for a longer period, and although the contemplation under German duress could scarcely be regarded as calm, the survey and recapitulation have been made. We may look forward eagerly to the new lines of investigation that his study will stimulate in himself and his pupils.

Particularly original is the treatment of such questions as: anaphylaxis; the nature of specificity; partial immunity; the relations of phagocytes to parenteral digestion; auto cytotoxins in their relation to chronic disease; and many others. It is to be hoped that a translation will soon render this valuable and authoritative work more readily accessible to the English speaking world.

—F. P. G.

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### *A Research Information Bureau*

THE National Research Council has established a Research Information Service as a general clearing house and informational bureau for scientific and industrial research. This "Service" on request supplies information concerning research problems, progress, laboratories, equipment, methods, publications, personnel, funds, etc.

Ordinarily inquiries are answered without charge. When this is impossible because of unusual difficulty in securing information, the inquirer is notified and supplied with an estimate of cost.

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Requests for information should be addressed, Research Information Service, National Research Council, 1701 Massachusetts Avenue, Washington, D. C.

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### *Epidemiology of Tuberculosis\**

COLONEL BUSHNELL, who so long and so successfully conducted the army tuberculosis hospital at Fort Bayard, N. M., and who had charge of the tuberculosis division in the Surgeon General's Office during the War, has given us a most valuable and readable book on tuberculosis. He has collected a great deal of information along lines which hitherto has not received adequate attention. Bushnell divides the peoples of the world into tubercularized and non-tubercularized races. In the former, tuberculosis is a chronic, slowly progressive, relatively benign disease. In nontubercularized races, it is an acute, epidemic, highly fatal disease. Certain localities, especially in tropical and semitropical countries, have had the reputation of being free from tuberculosis, but when an attempt has been made to use these places as resorts for those afflicted with this disease the natives have quickly developed the disease and supplied a very high death rate. In our own country we have in large numbers, so far as races are concerned, the white, the negro, and the Indian. Bushnell shows, especially by statistics of deaths in Charleston, S. C., that before the Civil War the tuberculosis death rate among whites and blacks was about the same. He concludes from this that even then the negro race was already tubercularized. Immediately following the Civil War the death rate among negroes rapidly increased and soon exceeded, and has continued to exceed, the death rate from this disease among the whites. The great increase in the death rate from tuberculosis among the blacks after the Civil War is attributed by Bushnell to poverty and bad housing conditions. He says: "The facts adduced as to the tuberculosis of the negro justify the following conclusions: the negroes as a race in the United States have long been in contact with the virus of tuberculosis. They are probably as well or nearly as well tubercularized as the white race. This is shown by the fact that when they were slaves, when their masters gave regular employment, provided food and to some extent looked after their health, their tuberculosis rates differed little from those of the whites. When their emancipation thrust them unprepared into the struggle for existence their sufferings and errors are revealed in an enormously increased mortality not only from tuberculosis but from other diseases."

Bushnell is quite certain that tubercularized races acquire a certain degree of immunity to the disease. This immunity is in no sense inherited, but must be acquired by each individual, and the danger lies in the acquisition of this im-

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\*A Study in the Epidemiology of Tuberculosis with Especial Reference to Tuberculosis of the Tropics and of the Negro Race by George E. Bushnell, Ph.D., M.D., William Wood & Co., New York, 1920.

munity. He says: "Civilized man can never escape the dangers of infection with the tubercle bacillus. But if we did escape the dangers of infection, we should also lose the benefits of tubercularization. Supposing that with extraordinary energy and sagacity we banish all tuberculosis from our town and rear an absolutely uninfected group of children. Having passed childhood under the irksome restrictions that would be necessary, the time must come when they shall be permitted to enter the outside world, for the fear of disease cannot remain the paramount consideration during life. As soon as, now adults or adolescents, they leave the sheltering confines of their native town, they will be exposed to the dangers of primary tuberculous infection and that at an age when the world beckons most invitingly and when prudence is least developed. In fact they would be in a hardly less dangerous situation than the tropical native when he first enters a civilized community. Prophylaxis has simply resulted in exchanging the danger of a chronic and usually relatively benign infection for the danger of an acutely fatal infection."

Bushnell is of the opinion that our Indians are just now becoming tubercularized and that in consequence of this the birth rate is now exceeding the death rate and the Indian promises to not disappear from the earth but to multiply and reproduce his kind. In 1891 an effort was made to try the Indian as a soldier. Authorization was obtained for eight troops of Indian Cavalry and nineteen companies of Indian Infantry. In the years immediately following, over eight hundred Indians were enlisted in the army and made up exclusively Indian organizations, but on account of the wide and deadly prevalence of tuberculosis among them this plan was given up.

It has been proposed that all children sent to the Indian schools should be tested with the tuberculin test and that those giving response to this test should not be sent to the schools. Bushnell comes to an opposite opinion and says: "In the writer's judgment, children should not be allowed to attend the larger schools unless they have a positive skin reaction to tuberculin. There is danger here that those who react positively may be on the point of breaking down with manifest tuberculosis of severe type, so that long railway journeys will lead to disaster. We would therefore make the further suggestion that no Indian child be sent to boarding school unless he is in apparently good health, shows at least no marked glandular involvement, gives no physical signs of tuberculosis of the lungs and has been positive for the Pirquet reaction for at least one year. In other words, a tuberculous vaccination should be required as well as a vaccination against smallpox."

Bushnell is of the opinion that we should give special attention to the protection of our children, and this attention should consist in endeavoring to prevent massive infection among them. There is only one logical conclusion to Bushnell's views and he does not hesitate to draw it—we must find some method of vaccination against this disease. We quite agree with the author that the greatest hope in the eradication of tuberculosis lies in the possibility of successful vaccination.

—V. C. V.

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## ORIGINAL ARTICLES

### COMPARATIVE VALUES OF COMPLEMENT-FIXATION METHODS IN SYPHILIS\*

By HOWARD D. MCINTYRE, M.D., EMERSON A. NORTH, M.D., AND AURELIA P.  
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#### PRELIMINARY NOTE

IN 1898, Bordet discovered the hemolysins. Three years later, only nineteen years ago, he published the first account of complement fixation which later became known as the "Bordet-Gengou phenomenon." Speaking in terms of the side-chain theory of Ehrlich, the phenomenon of complement fixation depends upon the fact that complement, a substance present in greater or less quantities in the serum of all animals, will be bound or fixed, in the presence of an antigen and its specific antibody or amboceptor, a more or less firm union of the three resulting.

Bordet's first experiments were carried out using the following reagents: As antigen, a suspension of *Bacillus pestis* in normal sodium chloride solution, guinea pig's serum for complement. The two were placed in a test tube containing the serum from a horse which had been immunized against *Bacillus pestis*. The three were incubated for one hour, after which the hemolytic system consisting of the serum of a guinea pig which had been immunized against rabbit's erythrocytes, and a quantity of rabbit's erythrocytes were added. As the complement had been fixed in the first incubation, no hemolysis resulted.

Bordet's work was soon corroborated by Ehrlich and Morgenroth. Widal and Lesourd about this time made use of the reaction in the diagnosis of typhoid using *baeillus typhosus* as antigen. This was the first practical use made of the reaction.

\*Acknowledgment is due the other members of the medical staff for their clinical cooperation.

Although the work of Bordet and of Ehrlich coincided from an experimental standpoint, theoretically they held quite different views as to the underlying mechanism of the reaction of complement binding or fixation.

Bordet held and still holds that the antibody unites directly with the antigen and serves to sensitize it and prepare it for direct union with the complement as a mordant aids in the penetration of a dyestuff. Mordants are substances which are capable of attaching themselves to the substance to be dyed and subsequently to the dye itself. Substances of this kind are tannic acid (for basic dyes) and colloidal hydroxides (for acid dyes) such as the hydroxides of aluminium, tin, iron, and chromium. For example, the cloth to be dyed is treated with aluminium sulphate and acquires either by absorption or feeble combination, or both, a certain amount of the hydroxide; the fabric is then boiled in water with an acid dye (say Turkey red or alizarine which is a very slightly soluble acid,  $C_{14}H_8O_4$ ) which unites with the hydroxide which has already united with the cloth. If we substitute the terms antigen for cloth, antibody for mordant, complement for dye, we can readily visualize in physico-chemical terms, Bordet's concept of the process of complement fixation.

Ehrlich, on the other hand, postulates the existence in cells of the body, of certain molecular groups which he calls receptors, which have the power to anchor or bind substances useful to them. Or the substance bound may be toxic to the cell and the cell may die or may be stimulated to the production of other receptors. These receptors, according to Weigert's law of overcompensation, ("overproduction theory"), are produced in excess and thrown into the blood stream. Ehrlich believes that these receptors or amboceptors play an all important rôle in complement fixation. According to him, the complement does not unite directly with the antigen but indirectly through the medium of the amboceptor which possesses a cytophile group which unites it with the antigen and a complementophile group which unites it with the complement.

In 1905, Wassermann and Bruck found that extracts of bacteria could be used in place of bacterial emulsions such as Bordet had used, also that extracts of tissue rich in bacteria might be used as antigen. Wassermann made use of the reaction in the diagnosis of tuberculosis, using as antigen lung tissue from tuberculous patients.

At this time the attention of the scientific world was drawn to syphilis, by the discovery of the *Spirocheta pallidum* by Schaudinn and Hoffman. In co-operation with Neisser, Wassermann and Bruck, May 10, 1906, made use of the reaction working with the serum from luetic monkeys using extracts of condylomata, luetic placenta, and so forth; as antigens. Just fourteen days later, Detre published his paper. He performed the reaction on six luetic and four normal persons, using tonsillar exudate, extract of luetic papules and pancreas as antigens. He obtained two positive reactions from the six luetic persons and all negative reactions from the normals.

Wassermann's first work on two hundred and seventy-five sera from luetic patients showed only nineteen per cent of positive reactions. This was due to the fact that elaborate controls and titrations now in use were lacking.

Following these pioneer papers many others appeared rapidly until the work done on serology has really assumed enormous proportions. At first the

Wassermann reaction was thought to be specific, that is, that each antigen produced its specific amboceptor in the blood stream of an immunized animal. However, the side-chain theory which Ehrlich elaborated to substantiate this view received a rude jolt, when in 1906 Citron showed that an extract from normal human red blood cells constitutes a fairly good antigen yielding positive reactions in about 80 per cent of luetic patients. In 1907, Weygandt obtained positive reactions on luetic sera using an extract of normal spleen as antigen. Müller and others used alcoholic extract of guinea pig heart the same year. These results directed attention to the fact that the antigen was probably a lipid, and following this conception much work was done by many observers using sodium glycocholate and taurocholate, cholesterol, lecithin, vaselin, and so forth as antigens.

The investigators were so numerous, the technic employed so different, the results so antagonistic and confusing, that the reaction fell into disrepute, its opponents claiming that it yielded positive reactions in tuberculosis, leprosy, scarlatina, in fact every infection besetting the human race. The painstaking efforts of honest workers, however, have shown pretty conclusively that we get little or no cross fixation in any diseases with the possible exception of frambesia (yaws) and leprosy. To this feature we will return later when we speak of our own experience with tuberculosis and other infections.

Coming now to our own work, it is our intention to outline the clinical and laboratory conditions existing in our institution calling attention to certain features of the work which we think might render our report valuable to other workers. Although the number of sera examined to date is not so great as from some of the larger laboratories of the country, we think that our results are fairly conclusive because of the following conditions existing.

(1) We have very close cooperation between the laboratory workers and the clinicians, the details of this paper could never have been worked out without the hearty cooperation of the clinical staff which involved much extra work on their part, such as frequent consultation, repeated physical examination, and clinical observation.

(2) We have the patients under observation for a considerable time, enabling us to arrive at more accurate clinical diagnoses than we could ever hope to do otherwise. In every doubtful case several blood examinations were made at different times.

(3) The sera to be examined are all drawn on the day to be examined, by the same persons, using as nearly as possible the same technic each time. Nearly all sera examined from our hospital are examined within the first twenty-four hours after being drawn.

Early in our work our laboratory procedure consisted in subjecting each serum examined to the so-called classical Wassermann, together with the Gradowohl<sup>1</sup> modification of the Hecht test, using an alcoholic extract of beef heart as antigen. We soon discovered, however, that these procedures at best yielded a rather low percentage of positive reactions in patients in whom we had no difficulty in finding the various clinical pictures of lues affecting the central nervous system. This was due to the facts that the patients were suffering from late

lues, also nearly all of them had been subjected to vigorous treatment before being admitted to Longview.

After reviewing the various methods of complement fixation in lues, we decided to subject all sera to the following tests for the purpose of ascertaining which would be of the most value to us in our work.

(1) The Wassermann reaction, employing complement fixation in the water-bath at  $37.5^{\circ}$  C., using two antigens, one a plain alcoholic extract of beef heart properly titrated, the other the same extract reinforced with cholesterol, also properly titrated.

(2) The same technic as in (1) with the exception that complement fixation was carried out in the ice box at  $2^{\circ}$  C. for a period of ten hours, observing certain precautions to be alluded to later.

(3) The Gradwohl modification of the Hecht test with some slight alterations to be described.

If we investigate the results of other workers with the classical Wassermann with complement fixation in the water-bath, we find that the best figures give positive reactions in from 80 to 90 per cent of primary lues. In secondary lues, Kolmer reports 100 per cent, Boas 100 per cent, Craig 96 per cent positive reactions in the untreated cases. In untreated tertiary lues the reaction yields from 90 to 100 per cent positive results, while in the treated cases of tertiary lues the percentage of positive reactions ranges from 65 to 75 per cent.

Turning now to the results of the classical Wassermann with complement fixation in the ice box, together with the use of cholesterolized antigen we find rather conflicting reports. So far as the writer can learn, Jacobstahl<sup>2</sup> (1910) was the first to employ this method, fixing the complement in the ice box at  $4^{\circ}$  C. for a period of one and one-half hours. McNeil<sup>3</sup> was the first American to employ the method.

Concerning cholesterolized antigen Owen and Martin<sup>4</sup> state, "the published figures of positive reactions (with cholesterolized antigen) run as high as 10 to 20 per cent in patients who do not have lues." This most decidedly does not coincide with our experience. We have never, except in one instance, to be discussed later, obtained a positive reaction using the cholesterolized antigen on any patient on whom the diagnosis of lues could not be made either from the clinical history, the physical findings, or both.

Ottenberg,<sup>5</sup> Smith and McNeil<sup>6</sup> state that the cholesterolized antigen cannot be used with safety in the Wassermann with complement fixation in the ice box. Our experience will not enable us to concur in this conclusion.

#### THE HECHT-GRADWOHL TEST

The principle upon which this reaction works, that is, making use of the native complement and hemolysins in the blood serum, was first suggested by Tschernogouboff who employed guinea pigs' erythrocytes in the performance of the reaction, neglecting, however, the quantitative relationships of the test. Hecht also made use of the test using sheep's erythrocytes. His technic, however, lay open to the same objections as that of Tschernogouboff. It remained for Gradwohl to place the test on a reliable basis.



We employ practically the same method as outlined by Gradwohl\* with the exception that we use fewer tubes (five to be exact) in the determination of the hemolytic index; we use three units, two units, and one unit of antigen in the test tubes of the back row, also we add an amount of red cells in the last incubation equivalent to one-half the amount of the hemolytic index. These alterations were suggested by Kolmer of Philadelphia. We have, furthermore, employed three types of antigen in our work, (1) the acetone insoluble as originally used by Gradwohl, also (2) a plain alcoholic extract of beef heart, and (3) a cholesterolized extract. The acetone insoluble is the most satisfactory. It is, however, more difficult of preparation. (For proof of this see tables that follow.)

With his technic Gradwohl has obtained fifteen per cent more positive reactions than with the classical Wassermann test. In his earlier paper he regards this as the best check on the Wassermann reaction of Neisser, Bruck, and Wassermann. In a later paper he concludes that the Hecht-Gradwohl test is not only a check, but, if positive, is diagnostic of lues. He states that he has never obtained a positive Wassermann reaction with a negative Hecht-Gradwohl test on the same serum, also that a doubtful Wassermann test should be thrown out in the presence of a negative Hecht-Gradwohl reaction on the same serum. He finds that the Hecht-Gradwohl test can be performed on 98 per cent of sera examined, his average hemolytic index is .5 c.e.

In the main our results coincide with those of Gradwohl. However, we find that there are some sera which may show complete inhibition in the first two tubes containing antigen, with only a partial inhibition in the third, or only the first tube may show complete inhibition with partial or no inhibition in the last two antigen containing tubes, or all tubes containing antigen may show a partial inhibition, the control being clear. In this we are in agreement with A. J. Blaivas.<sup>7</sup> We may say here that those sera showing partial inhibition, with very few exceptions, were found by extensive clinical observation to have come from luetic patients. Furthermore, it is our opinion either with the Wassermann or Hecht-Gradwohl technic, if the technic has been accurate, sera showing partial inhibition should always be regarded with suspicion and it has been our experience that such sera were found on clinical observation to have come from patients with lues in one form or another. It is our custom to subject such sera to repeated examinations as it has been shown that the amount of antiluetic amboceptor in a patient's serum may vary from day to day in the same patient.

It has been our experience that the positive Wassermann reaction will give way to a negative Wassermann reaction under treatment, while the Hecht-Gradwohl test may remain strongly positive for some time afterward.

The reasons advanced as to why the Hecht-Gradwohl test is more sensitive than the Wassermann reaction are:

(1) There are two antiluetic amboceptors in serum from luetic patients, one is destroyed by inactivation, or heating to 56° C. as in the Wassermann test. As the serum is not inactivated in the Hecht-Gradwohl test this difficulty is obviated.

(2) By making use of the native complement and antishoop amboceptor in the patient's serum we avoid the excess of antishoop amboceptor over the com-

\*For the details of the technic see Gradwohl's original paper, Jour. Am. Med. Assn., 1914, p. 240.

plement which may be so great as to throw a positive reaction to the negative side when the hemolytic system is added in the final step of the Wassermann reaction.

Concerning our Wassermann technic, without going into great detail we would like to emphasize a few points.

(1) Sera should be examined within the first twenty-four hours after being drawn.

(2) Complement should not be drawn from the guinea pig until needed for titration. It is the custom in our laboratory never to use complement after it has been drawn twelve hours.

(3) The concentration of cholesterol in the antigen should never exceed 0.4 per cent.

Regarding the Wassermann test with complement fixation in the ice box, we believe that the period of fixation should not exceed ten hours, neither should the temperature exceed 2° C. We use this procedure as it is the optimum time and temperature as worked out by Ruediger.

The technic is the same as in the classical Wassermann test up to the point of complement fixation when the tubes are placed in the ice box for ten hours at 2° C. At the end of this time we again wash the cells if the supernatant fluid shows any tinge of red. We also titrate the complement and amboceptor once again to make sure that they have not changed in titer. Some workers have said that the reason the ice box method yields more positive reactions is that the complement deteriorated in the time allotted to this method. This is not true in the light of our work.

After the tubes have been removed from the ice box it is well to warm them

TABLE I

Comparing (1) the Wassermann Reaction with complement fixation in the water-bath at 37.5° C.

(2) the Hecht Gradwohl test, using the plain alcoholic extract of beef heart as antigen in 104 cases of lues involving the central nervous system.

Results (1) *The Wassermann test, complement fixation in the water bath at 37.5° C.*

	Number	Percentage
Positive	51	49.03
Borderline	7	6.73
Negative	46	44.23

(2)

*The Hecht Gradwohl test with plain antigen. Total number possible, 94.*

	Number	Percentage
Positive	56	59.57
Borderline	5	5.21
Negative	33	35.1
Impossible	7	

Number

Sera negative to the Wassermann test, but positive to the Hecht Gradwohl test .....	9
Sera doubtful to the Wassermann test, but positive to the Hecht Gradwohl test .....	6
Sera positive to the Wassermann test, but impossible to the Hecht Gradwohl test .....	7

in the water-bath before adding the hemolytic system, as also suggested by Ruediger (*loc. cit.*).

The hemolytic system is added and the tubes returned to the water-bath at 37.5° C. for thirty minutes and read as before. We usually do two readings, one at the end of the last incubation, and one when all the cells in the tubes showing inhibition have settled out. The final reading is the one used for permanent record. We do not, however, consider a doubtful reaction with the ice box method as diagnostic of lues, but we do consider a reaction of the four-plus

TABLE II

Comparing (1) The Wassermann reaction with complement fixation in the water bath at 37.5° C.

(2) the Hecht-Gradwohl reaction.

(a) Plain antigen and (b) plain antigen reinforced with cholesterol was used in both tests in 60 cases of lues involving the central nervous system.

Results

(1)

*The Wassermann test, using plain antigen, complement fixation in the water-bath at 37.5° C.*

	Number	Percentage
Positive	22	36.66
Borderline	4	6.66
Negative	34	56.66

(1')

*The Wassermann test, using cholesterolized antigen, complement fixation in the water-bath at 37.5° C.*

	Number	Percentage
Positive	25	41.66
Borderline	13	21.66
Negative	22	36.66

(2)

*The Hecht-Gradwohl test, using plain antigen. Total number of cases possible, 55.*

	Number	Percentage
Positive	28	50.9
Borderline	3	5.45
Impossible	5	
Negative	24	43.62

(2')

*The Hecht-Gradwohl test, using cholesterolized antigen in the same cases.*

	Number	Percentage
Positive	37	67.2
Borderline	3	5.4
Impossible	5	
Negative	15	18.4

Number

Sera negative to the Wassermann test, but positive to the Hecht-Gradwohl test with plain antigen .....	7
Sera doubtful to the Wassermann test, but positive to the Hecht-Gradwohl test with plain antigen .....	5
Sera negative to the Wassermann test, but positive to the Hecht-Gradwohl test with cholesterolized antigen .....	5
Sera doubtful to the Wassermann test, but positive to the Hecht-Gradwohl test with cholesterolized antigen .....	11
Sera positive to the Wassermann test, but impossible to the Hecht-Gradwohl test with both plain and cholesterolized antigen .....	4

variety as diagnostic of lues. We have never (with one exception) obtained such a reaction in a person who was not a luetic, but we have obtained three doubtful reactions with serum from patients in whom the diagnosis of lues could not be made.

Following are some tables compiled from data derived from examinations of over one thousand sera from patients admitted to Longview Hospital, together with four hundred and ten serum examinations on patients undergoing treatment for lues at the State Reformatory for Women, at Marysville, Ohio. Luetic cases only are considered in the accompanying tables. The remainder of the sera were from normal persons, that is, normal so far as could be determined by serologie and clinical examination.

The total number of sera from which Table I is made is 901. Only luetic cases are included in the accompanying tables.

The low percentage of positive reactions obtained in this as well as in our other series is due to the fact that nearly 100 per cent of all cases admitted to Longview Hospital have been subjected to long and vigorous treatment before the tests were performed. This fact accounts also for the considerable number of borderline or so-called doubtful reactions as serologie tests are less liable to be clear-cut positives or negatives in treated cases of lues than in untreated ones.

In Table II the percentage of positive reactions yielded by the Hecht-Gradwohl test over the Wassermann test is 10. The Hecht-Gradwohl test helped to a diagnosis of lues in 15 cases out of 104 where the Wassermann test was doubtful or negative. On the other hand, the Wassermann test helped to a diagnosis in 7 cases of lues in which the Hecht-Gradwohl test was impossible because of the absence of a hemolytic index.

TABLE III

- Comparing (1) The Wassermann test with complement fixation in the water-bath for thirty minutes at 37.5° C.  
 (2) The Wassermann test with complement fixation in the ice box for ten hours at 2° C.  
 (3) The Hecht-Gradwohl test  
 (a) Plain alcoholic extract of beef heart and (b) the same extract reinforced with cholesterol constituted the antigens used in each test. Sera from 62 patients suffering with neurosyphilis in one form or another were subjected to the above tests. Sera from 319 nonluetie persons examined in the same series showed uniformly negative reactions to all tests.

Results (1)

*The Wassermann test with complement fixation at 37.5° C. for thirty minutes in the water-bath with plain antigen in 62 cases of lues.*

	Number	Percentage
Positive	17	27.41
Borderline	8	12.90
Negative	37	59.67

(1')

*The Wassermann test with complement fixation at 37.5° C. with cholesterolized antigen in 62 cases of lues.*

	Number	Percentage
Positive	23	37.09
Borderline	12	19.35
Negative	27	43.55

(3)

*The Hecht-Gradwohl test with plain antigen. Total number of cases possible, 55.*

	Number	Percentage
Positive	25	45.45
Borderline	8	14.54
Impossible	7	
Negative	22	40.00

(3')

*The Hecht-Gradwohl test with cholesterolized antigen in same cases.*

	Number	Percentage
Positive	32	58.18
Borderline	4	7.27
Impossible	7	
Negative	19	34.54

Number

Sera negative to the water-bath Wassermann test using the plain antigen but positive to the Hecht-Gradwohl test using the plain antigen ..... 5

Sera doubtful to the water-bath Wassermann test using plain antigen but positive to the Hecht-Gradwohl test using plain antigen ..... 5

Sera negative to the water-bath Wassermann test but positive to the Hecht-Gradwohl test, cholesterolized antigen being used in both tests ..... 4

Sera doubtful to the water-bath Wassermann test, using cholesterolized antigen but positive to the Hecht-Gradwohl test using the same antigen 10

(2)

*The Wassermann test with complement fixation in the ice box at 2° C. for ten hours with plain antigen in 62 cases of lues.*

	Number	Percentage
Positive	53	85.48
Borderline	5	8.06
Negative	4	6.45

(2')

*The Wassermann test with complement fixation in the ice box at 2° C. for ten hours with cholesterolized antigen in 62 cases of lues.*

	Number	Percentage
Positive	61	98.38
Borderline	0	
Negative	1	1.61

Twenty sera reacted negatively using two antigens in the Hecht-Gradwohl test, which yielded positive reactions to the Wassermann test, employing complement fixation in the ice box at 2° C. for a period of ten hours. Seven sera had no hemolytic index, hence the Hecht-Gradwohl test was not done. However, all of these sera showed a positive reaction to the Wassermann test employing complement fixation in the ice box.

In this series the Hecht-Gradwohl test, using the plain antigen, helped to a diagnosis of lues in twelve cases where the Wassermann test was either doubtful or negative. The Hecht-Gradwohl test, using cholesterolized antigen, helped to a diagnosis of lues in sixteen cases where the Wassermann test was negative. On the other hand, the Wassermann test made a positive diagnosis in four cases where the Hecht-Gradwohl test was impossible.

The interesting point observed in this series is the high percentage of positive reactions obtained with the Wassermann test when complement fixation in the ice box is employed. Under these conditions the Wassermann reaction yielded positive reactions in twenty cases where the Hecht-Gradwohl test was

negative, and a positive reaction in seven cases where the Hecht-Gradwohl was impossible. In other words twenty-seven cases of lues out of the sixty-two in this series would have been undiagnosed serologically if reliance had been placed upon one test alone.

We must bear in mind, however, that this does not mean that the Hecht-Gradwohl test would not have been positive had complement fixation been carried out in the ice box. We are at present working on this phase of the work and our results so far show that if complement fixation in the Hecht-Gradwohl test is carried out in the ice box at 2° C. for ten hours the Wassermann and Hecht-Gradwohl tests agree in practically 100 per cent of all cases. This feature will be dealt with in a future study.

TABLE IV

Comparing (1) The Wassermann reaction with complement fixation at 37.5° C. for 30 minutes,  
 (2) The Wassermann reaction with complement fixation at 2° C. for 10 hours,  
 (3) The Hecht-Gradwohl test  
 . (a) Plain antigen and (b) cholesterolized antigen used in all tests in 20 cases of latent lues.

Results (1)

*The water-bath Wassermann test using plain antigen.*

	Number	Percentage
Positive	2	10
Borderline	1	5
Anticomplementary	0	
Negative	17	85

(1')

*The water-bath Wassermann test using cholesterolized antigen.*

	Number	Percentage
Positive	2	10
Borderline	1	5
Anticomplementary	0	
Negative	17	85

(3)

*The Hecht-Gradwohl test, plain antigen, 18 cases possible.*

	Number	Percentage
Positive	5	27.77
Borderline	1	5.55
Impossible	2	
Negative	12	66.66

(3')

*The Hecht-Gradwohl test, cholesterolized antigen, 18 cases.*

	Number	Percentage
Positive	9	50
Borderline	0	
Impossible	2	
Negative	9	50

(2)

*The ice-box Wassermann test, plain antigen, 19 cases possible.*

	Number	Percentage
Positive	15	78.95
Borderline	1	5.26
Anticomplementary	1	
Negative	3	15.79

(2')

*The ice-box Wassermann test, cholesterolized antigen, 19 cases.*

	Number	Percentage
Positive	17	89.47
Borderline	1	5.26
Anticomplementary	1	
Negative	1	5.26

In many of these cases the diagnosis of lues was unsuspected but being led by the positive serology, the diagnosis was later established by history and close physical examination.

TABLE V

Comparing (1) The water-bath Wassermann test, using plain antigen in 145 cases of lues under treatment.  
 (2) The Hecht-Gradwohl test, using plain antigen in 126 possible cases of lues under treatment.

Results

(1)

*The water-bath Wassermann test in 145 cases of treated lues.*

	Number	Percentage
Positive	9	6.20
Borderline	3	2.06
Negative	133	91.72

(2)

*The Hecht-Gradwohl, plain antigen, 126 cases possible, treated lues.*

	Number	Percentage
Positive	26	20.63
Borderline	4	3.17
Impossible	19	
Negative	96	76.19

The Hecht-Gradwohl test yielded 14 per cent more positive reactions in this series of treated cases than did the Wassermann test.

TABLE VI

Comparing (1) The Wassermann test, complement fixation in the water bath at 37.5° C. for 30 minutes.  
 (2) The Wassermann test, complement fixation in the ice box at 2° C. for 10 hours.  
 (3) The Hecht-Gradwohl test.  
 (a) Plain antigen and (b) cholesterolized antigen used in all tests of 157 cases of lues under treatment.

Results

(1)

*The water-bath Wassermann test using plain antigen in 157 cases.*

	Number	Percentage
Positive	12	7.64
Borderline	5	3.18
Negative	140	89.17

(1')

*The water-bath Wassermann test using cholesterolized antigen.*

	Number	Percentage
Positive	17	10.82
Borderline	15	9.55
Negative	125	79.61

(3)

*The Hecht-Gradwohl test using plain antigen, 128 cases possible.*

	Number	Percentage
Positive	25	19.53
Borderline	3	2.34
Impossible	29	
Negative	100	78.12

(3')

*The Hecht-Gradwohl test using cholesterolized antigen in 128 cases.*

	Number	Percentage
Positive	36	28.12
Borderline	14	10.93
Impossible	29	
Negative	78	60.93

(2)

*The ice-box Wassermann test, plain antigen in 157 cases.*

	Number	Percentage
Positive	69	43.94
Borderline	18	11.46
Negative	70	44.58

(2')

*The ice-box Wassermann test, cholesterolized antigen in 157 cases.*

	Number	Percentage
Positive	83	52.86
Borderline	17	10.82
Negative	57	36.30

The data in Table VII serves to show that even using the acetone-insoluble antigen in the Hecht-Gradwohl test does not yield as high a percentage of positive reactions as does the Wassermann reaction employing complement fixation in the ice-box. However, this does not in any way militate against the earlier conclusions of Gradwohl, neither does it detract in the slightest degree from the efficiency of his test. We may repeat here that when the two tests are carried out with complement fixation in the ice chest in both, the results agree in about 100 per cent of the cases.

Our tables serve to show that methods of complement fixation in the ice chest are superior to methods of complement fixation in the water-bath. Why is this so? We may begin our answer to this question by stating that when a serologist uses only one method of complement fixation in lues he neglects the quantitative relationship of the Wassermann reaction. It is true that in the untreated secondary cases, or in cases of lues reacting strongly to the germ of syphilis, the original Wassermann technic will yield positive reactions in about 100 per cent, even though the weakest antigen, such as the plain alcoholic extract of beef heart, is used. In latent lues, treated lues and lues involving the nervous system such a procedure will yield at best a low percentage of positive reactions, whereas the more delicate methods will yield a much higher percentage of positive reactions. It is just as important, and infinitely more difficult, to establish a diagnosis in the latent types of lues as in the frank types, hence the need of more delicate reactions such as the Hecht-Gradwohl test and ice box fixation tests.



TABLE VII

- Comparing (1) The water-bath Wassermann test, using plain and cholesterolized antigen.  
 (2) The ice-box Wassermann test, using plain and cholesterolized antigen.  
 (3) The Hecht-Weinberg-Gradwohl test, using the cholesterolized and the acetone insoluble antigen.

Of three hundred sera thus examined twenty-two were from patients suffering with neurosyphilis, seventy-seven were from cases of lues under treatment, the remainder were negative both clinically and serologically.

Results in twenty-two cases of neurosyphilis.

(1)

*The water-bath Wassermann test using plain antigen.*

	Number	Percentage
Positive	13	59.09
Borderline	2	9.09
Negative	7	31.81

(1')

*The water-bath Wassermann test using cholesterolized antigen.*

	Number	Percentage
Positive	16	72.72
Borderline	2	9.09
Negative	4	18.18

(2)

*The ice-box Wassermann test using plain antigen.*

	Number	Percentage
Positive	21	95.45
Borderline	1	4.54
Negative	0	

(2')

*The ice-box Wassermann test using cholesterolized antigen.*

	Number	Percentage
Positive	22	100
Borderline	0	
Negative	0	

(3)

*The Hecht-Gradwohl test, cholesterolized antigen in 21 possible cases.*

	Number	Percentage
Positive	15	71.42
Borderline	3	14.28
Impossible	1	
Negative	3	14.28

(3')

*The Hecht-Gradwohl test, acetone-insoluble antigen in 21 possible cases.*

	Number	Percentage
Positive	17	80.95
Borderline	3	14.28
Impossible	1	
Negative	1	4.76

Results in seventy-seven cases of treated lues.

(1)

*The water-bath Wassermann test, plain antigen, 77 cases.*

	Number	Percentage
Positive	1	1.29
Borderline	3	3.89
Negative	73	94.81

(1')

*The water-bath Wassermann test, cholesterolized antigen.*

	Number	Percentage
Positive	7	9.09
Borderline	2	2.59
Negative	68	88.31

(2)

*The ice-box Wassermann test, plain antigen, 77 cases.*

	Number	Percentage
Positive	22	28.57
Borderline	10	12.98
Negative	45	58.44

(2')

*The ice-box Wassermann test, cholesterolized antigen.*

	Number	Percentage
Positive	36	46.75
Borderline	5	6.49
Negative	36	46.75

(3)

*The Hecht-Gradwohl test, cholesterolized antigen, 68 cases possible.*

	Number	Percentage
Positive	6	8.82
Borderline	16	23.53
Impossible	9	
Negative	46	67.64

(3')

*The Hecht-Gradwohl test, acetone insoluble antigen, 68 cases.*

	Number	Percentage
Positive	16	23.53
Borderline	9	13.23
Impossible	9	
Negative	43	63.23

The main reason why the ice box method of fixation is superior to the water-bath method is that the former test will detect the antiluetic reacting substances in the blood stream in higher dilutions than will the latter. To prove this one has but to perform the following simple experiment.

The serum used in this experiment yielded the following results when subjected to our routine tests using .1 c.c. of serum:

Water bath Wassermann test	Results
Plain antigen . . . . .	+++
Cholesterolized antigen . . . . .	+++
Ice box Wassermann test	
Plain antigen . . . . .	+++
Cholesterolized antigen . . . . .	+++
Hecht Gradwohl test	
Plain antigen . . . . .	Positive (a <sup>+</sup> b <sup>+</sup> c <sup>+</sup> d <sup>+</sup> )
Cholesterolized antigen . . . . .	Positive (a <sup>+</sup> b <sup>+</sup> c <sup>+</sup> d <sup>+</sup> )
Acetone Insoluble antigen . . . . .	Positive (a <sup>+</sup> b <sup>+</sup> c <sup>+</sup> d <sup>+</sup> )

It will be readily seen that this serum contains a high concentration of the reacting substances of lues.

This serum was diluted as indicated in the diagram in two sets of tubes and

the Wassermann reaction employing water-bath fixation was performed on one set of tubes; the ice chest method of fixation was performed using the other set. Plain and cholesterolized antigens were used. The results of the water-bath fixation are recorded in the upper portion of the tube in the diagram, the results of the ice chest fixation in the lower. It is readily seen that the ice box fixation method using cholesterolized antigen yields a  $++$  reaction in a higher dilution than does either the plain antigen in the ice chest or both the antigens in

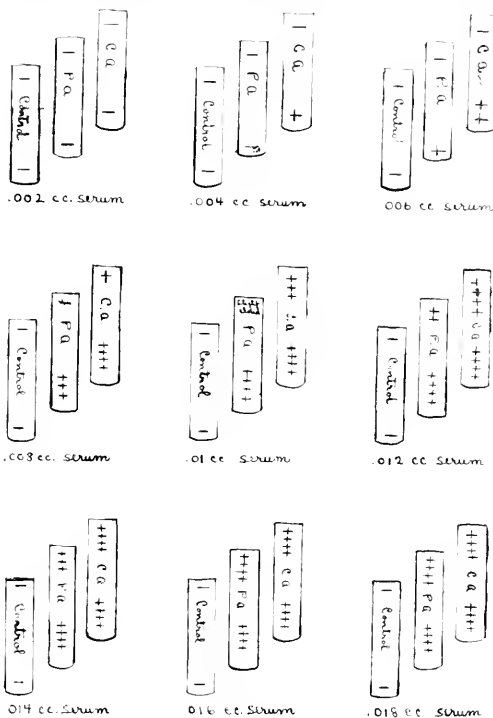


Fig. 1.

the water-bath. (Note results in the set of tubes containing .008 c.c. of patient's serum.)

One finds that when working with sera which react strongly to the original Wassermann test complement fixation takes place with great rapidity whereas with serums containing less of the reacting substances complement fixation takes place much more slowly. In this respect complement fixation follows the laws of adsorption, a point in favor of Bordet's theory concerning the phenomenon. This fact also points to an explanation of why the longer period of ice box fixation method using cholesterolized antigen yields a  $++$  reaction in a higher

period of complement fixation in the water-bath. The main reason why long periods of fixation cannot be carried out in the water-bath is that complement deteriorates rapidly in the water-bath.

#### SUMMARY

1. Cholesterolized antigen properly prepared and titrated yields from 10 to 15 per cent more positive Wassermann reactions on luetic sera than does the plain antigen. We consider it a perfectly safe antigen to employ in the Wassermann reaction with complement fixation in the ice box at 2° C. for a period not longer than ten hours observing the precautions outlined in this paper. We have obtained but one positive reaction employing such methods in which the clinical findings, the history, or both, did not justify a diagnosis of lues. We may say here that we still have this patient under observation and there is a great possibility which may be later established that this patient has had lues.

2. The Hecht-Gradwohl test when positive in the temperate zone is diagnostic of lues. It will yield 15 per cent more positive reactions on luetic sera than does the classical Wassermann reaction. It may be employed in from 95 to 98 per cent of fresh sera (not over forty-eight hours old). It *does not* yield false positive results in tuberculousis.

The Wassermann test employing complement fixation in the ice box at 2° C. will yield a much higher percentage of positive reactions than does the Hecht-Gradwohl test employing complement fixation in the water-bath. With complement fixation under the same conditions, however, the tests practically agree.

The three serologic reactions appear in the serum and disappear under treatment in the following order:

The ice box Wassermann reaction is the first to appear positive, the Hecht-Gradwohl test follows, the water-bath Wassermann reaction appearing last. Under treatment the water-bath Wassermann reaction disappears first, the Hecht-Gradwohl reaction next, the ice box Wassermann reaction last.

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## PRIMARY LYMPHOSARCOMA OF THE STOMACH. A REPORT OF TWELVE CASES

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PRIMARY lymphosarcoma of the stomach compared with carcinoma of the stomach occurs in the proportion of 1 to 68 according to the records of the Mayo Clinic. But few cases are reported. Ruppert, in 1912, collected twelve cases from the German literature, to which he added one. A few cases have appeared from time to time in American, English, and French literature.

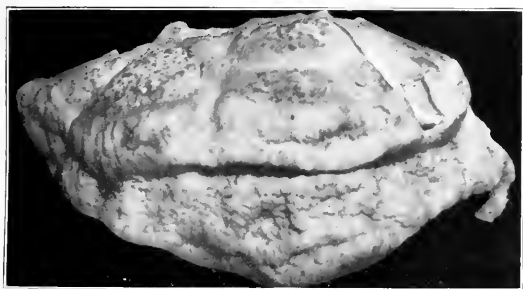


Fig. 1.—(a190100). Lymphosarcoma of the stomach. The rolled-edge border gives it the appearance of a mushroom lying on the gastric mucosa.

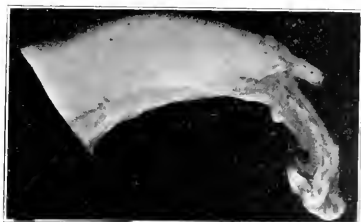


Fig. 2.—(a190100). Section of lymphosarcoma shown in Figure 1. The neoplasm has pushed up the mucosa and invaded the musculature.

In our series of twelve cases of lymphosarcoma of the stomach observed at the Mayo Clinic from January 1, 1913, to December 1, 1920, the average duration of clinical symptoms was six and eight hundredths months. Eleven patients gave a history of loss of weight, nine of pain, seven of vomiting, and two of bleeding; two had histories suggestive of previous ulcer. The average age of the patients was forty-six and twenty-five hundredths years; the youngest patient was 16, and the oldest, 62. Eleven were males.

In seven cases the clinical diagnosis was carcinoma; in one ulcer; in one abdominal tumor, probably inflammatory; in one lesion of the stomach, probably malignant; in one pyloric obstruction; in one upper abdominal tumor, probably of the pancreas.

Resection was performed in six cases; in the other six the condition was found to be inoperable on exploration. The neoplasms were located for the most part in the pyloric portion of the stomach.

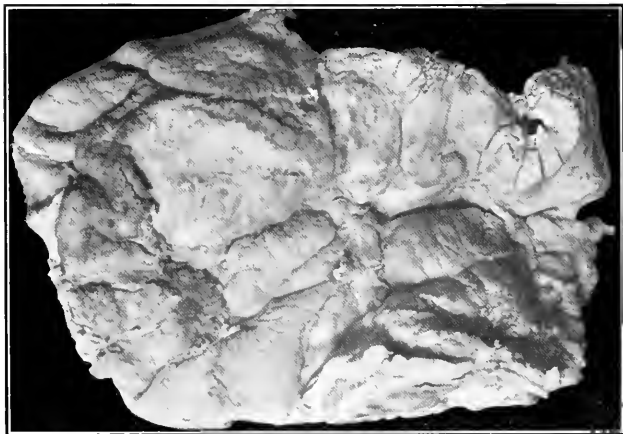


Fig. 3.—(a265434). Lymphosarcoma of the stomach associated with ulceration of the mucosa.



Fig. 4.—(a265434). Section of lymphosarcoma shown in Figure 3. The neoplasm has invaded the musculature; in one place it has extended through to the serosa.

#### PATHOLOGY

*Macroscopic Examination.*—On section of the stomach the rolled edged border of the neoplasm with its raised surface presents the appearance of a mushroom, lying in the folds of the normal mucosa (Figs. 1 and 2). The surface of this may or may not be ulcerated; the ulceration may be shallow or deep (Figs. 3 and 4). On section the neoplasm is fairly soft and resilient and pale

straw colored; it is limited largely to the submucosa, but here and there invades the musculature. The thickness of the wall of the stomach, including the tumor, may be as much as 3 cm. The growth in one of the cases in this series was very extensive. It appeared to extend directly through the muscle and serosa into the gastrocolic omentum. The surrounding lymph nodes were also extensively involved.

*Microscopic Examination.*—The tumor-cells massed about the glands of the gastric mucosa involve the entire space of the submucosa and extend down



Fig. 5.—(a316187). Low power photomicrograph showing the neoplastic cells massed around the gastric glands.

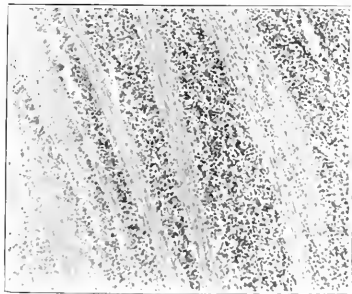


Fig. 6.—(a336365). Low power photomicrograph showing the neoplastic cells lying between the muscle fibers.

between the muscle fibers (Figs. 5, 6, and 7). The cells resemble for the most part those of the germ center with areas of normal lymphocytes scattered here and there. These cells are larger than normal lymphocytes, usually irregular and often contain prominent single nucleoli (one-eyed cells). The tissue is recognizedly lymphoid, but the absence of germ centers and the fact that the entire section is but a homogeneous structureless mass of cells with the exception of a few lymphocytes are particularly striking. In two of the six cases

in which a portion of the stomach was resected the lymph nodes were involved, and in one the serosa. In four of the six cases which proved to be inoperable at the time of exploration the adjacent lymph nodes were involved (Fig. 8).

#### RESULTS

Two of the six patients who had resections died of peritonitis following operation. One patient died four months after operation with a recurrence in the lower bowel, liver, and remainder of the stomach. One returned prae-

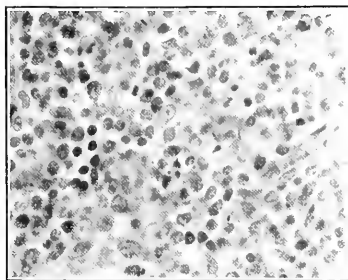


Fig. 7.—(c33363b5). High power photomicrograph of a neoplastic area shown in Figure 6.

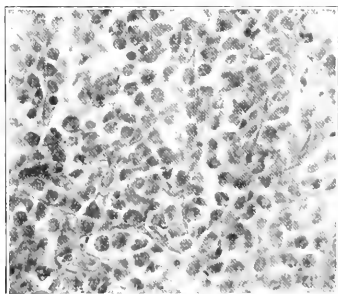


Fig. 8.—(a205800). High power photomicrograph of a lymphosarcomatous area of a lymph node lying close to the stomach.

tically five months after operation in poor condition; he had lost 20 pounds and he had a mass in the left epigastrium which probably was a recurrence. Another returned practically seven months after operation, apparently with a recurrence. The remaining patient was operated on too recently to be considered in the results. Four of the six patients who had explorations are dead. They lived for from six weeks to four months after exploration. Information has not been received from the remaining two patients.

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## THE ETIOLOGY OF ACUTE INFLAMMATIONS OF THE NOSE. PHARYNX AND TONSILS\*

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ST. LOUIS, MO.

(Continued from page 190.)

### VASOMOTOR REACTIONS TO CHILLING

TEMPERATURE changes in the skin and exposed mucous membranes were thus established as valid criteria, under the conditions of our experiments, of vasomotor tone. The graphs to follow actually show surface temperature changes; their significance is of alterations in blood supply. Obviously, fall in temperature means reflex vasoconstriction, a rise, vasodilation.

The vasomotor responses to chilling of a distant area of the body surface exhibited in the exposed skin of the forehead and of the mucous membrane of the soft palate and oropharynx, respiration and other conditions being carefully controlled as described above, may readily be made out from Fig. 4.

The two lower curves of this figure are composite graphs of four experiments of similar pattern. The points chosen for plotting are: the first and last readings of each experiment, the readings immediately before and after each change in experimental conditions, and the first point of maximum response of the mucous membrane to changed conditions; each point graphed is the average value of the corresponding point of the four experiments. Time is plotted on the horizontal axis, temperature on the vertical; the space between the ruled lines represents ten minutes on the abscissa, one-half degree centigrade on the ordinate. The character of the lines connecting the points gives the nature of the experimental conditions, as indicated in the legend.

While respiration is quiet and the subject wrapped, mucous membrane and skin temperatures remain constant. Deepening of respiration affects only inconsiderably the skin temperature, but causes a depression of mucous membrane temperature amounting to .68° C., which reaches its maximum after 25.4 minutes, and then, during the remaining 8.5 minutes before chilling begins, remains virtually constant.

The subject was chilled by unwrapping him and directing the draft of an electric fan against the lower thoracic region of his back. With the start of this process, a marked depression both of mucous membrane and of skin temperature begins. The maximum fall of mucous membrane temperature is 1.42° C., reached in 18.4 minutes. The synchronous point on the skin curve represents a drop of 1.73° C. The skin curve falls away a little more sharply

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The bibliography will appear at the end of the third section of this paper in the March number of the *Journal of Laboratory and Clinical Medicine*.

than the mucous membrane curve. However, even if the vasoconstriction in skin and mucous membrane were to follow an identical course, we should expect, on mechanical grounds, this difference in the curves; for the more exposed forehead would, of course, lose heat more rapidly than the mucosa of the palate and pharynx.

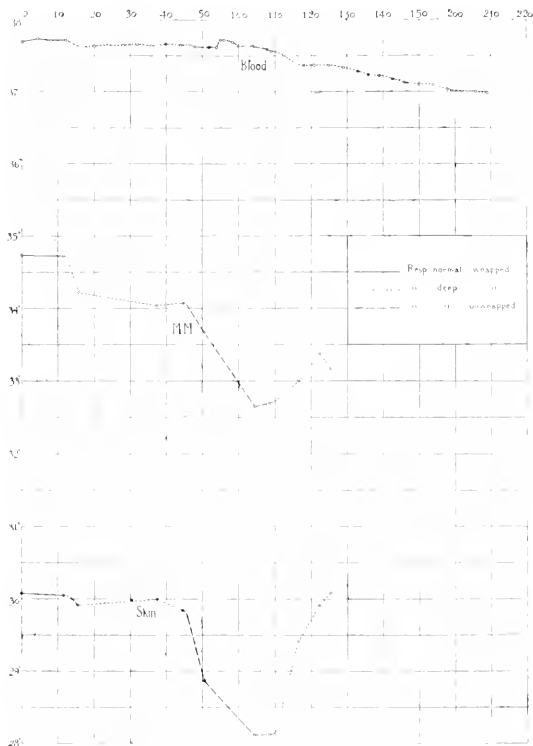


Fig. 4.—Effects of chilling body surface. Respiration controlled. Composite graphs of four experiments (temperatures of skin and mucous membranes of oropharynx and soft palate) and two experiments (blood temperature). Subject chilled by unwrapping, with draft of electric fan on back.

When it was seen the mucous membrane temperature had ceased to fall, the fan was turned off and the subject again wrapped. Here a disparity in the behavior of skin and mucous membrane vessels appears. The skin temperature climbs steeply and surmounts the level at which chilling began. The skin "reacts," as is commonly said. But the mucous membrane temperature rises only .73° C. Its maximum recovery is reached after 12.7 minutes and is .69° C. below the last point before chilling. During the remaining 3.5 minutes of observation, it falls .21° C.

No explanation in mere physics is to be found for the mucous membrane temperature remaining depressed; if its vessels returned to the same tone as before chilling, the temperature curve should return to control level. Yet this same incomplete recovery after chilling was noted in all the crucial experiments upon soft palate and pharynx, alike the early determinations with respiration uncontrolled and the final ones with respiration controlled. Measurement of the respiration records for the four experiments graphed in Fig. 4 was made, and the mean figure arrived at for respiratory amplitude before chilling differed from that after chilling by only a fraction of one per cent. In those experiments in which respiration was slightly deeper before as well as in those in which it was a few per cent deeper after chilling, the failure of the mucous membrane temperature to regain its former level is usually evident. We are forced to conclude, therefore, that the vasoconstriction and ischemia reflexly produced in the palatine and pharyngeal mucous membrane by chilling the body surface persist in part for some time at least. The skin in our experiments has tended more completely to regain its blood supply. The tonsils have tended to regain their blood supply even more completely than the skin; in some instances actually becoming hyperemic on rewarming (Grant, Mudd and Goldman, 1920).

The uppermost curve in Fig. 4 is a composite of two blood-temperature control experiments which followed the same pattern as the two lower curves. For discussion see above.

A few experiments, illustrating typical reactions in the several regions of the nose and throat, may now be briefly considered:

EXPERIMENT A.—M.M. thermopile on anterior half of *soft palate*. Skin thermopile on forehead. Respiration, 18 per minute. Mouth open, nose breathing. Thoracic and abdominal pneumographs. Room temperature 17.9° to 18.8° C.; time, 3:55 to 5:16 P.M.

Experiment A from 0:00 to 0:50, shows the typical picture described for the composite graph above save that the anterior palatine mucous membrane is not affected by deepening respiration. At 0:50 the subject's feet were exposed, wrapped in cold wet towels, and the electric fan turned on them. This seems to have been without effect upon the forehead, but was followed apparently by a slight depression, .29° C., of mucous membrane temperature. This effect was not sufficiently definite to warrant much emphasis, but is at least suggestive in view of the possible efficacy of wet feet in exciting colds.

Administration of amyl nitrite at 1:03, while the feet were still being chilled, was followed by a steep rise in mucous membrane and skin temperatures with quick return to normal. The break in the skin curve is meant to indicate that it may have risen above 30.50° C. in the interval, 1:02 to 1:08 during which no skin readings were made.

The uppermost curve in Fig. 5 is a blood temperature control.

EXPERIMENT B.—M.M. thermopile on posterior wall of *oropharynx*. Skin thermopile on forehead. Respiration, 18 per minute. Nostrils plugged with cotton, mouth breathing. Thoracic and abdominal pneumographs. Room temperature, 19.0° to 20.6° C.; time, 10:11 P.M. to 12:33 A.M.

The curve of Experiment B (Fig. 6) shows the pharyngeal mucous membrane reacting in essentially the manner indicated in the composite graph, and shown also for the palatine membrane in Experiment A. The difficulties in technic are considerably greater with the thermopile applied to the pharynx than to the palate, however, and the curve is never so smooth. The effects of moving the pharyngeal wall against the thermopile terminals by coughing, swallowing or clearing the throat, is shown at 0:27, 0:50.5 and 1:41. Presum-

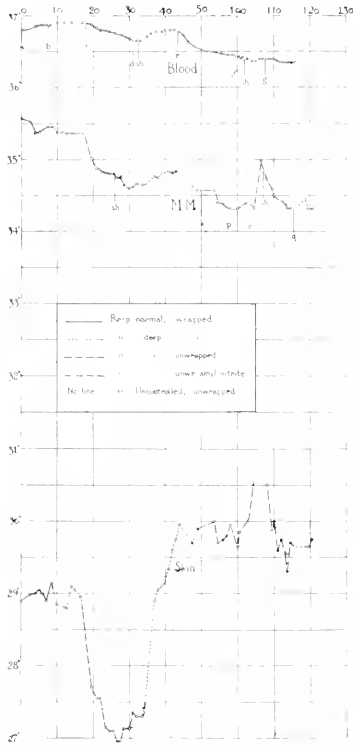


Fig. 5.—Chilling and amyl-nitrite effects. Experiment A (temperatures of skin and mucous membrane of soft palate) and Experiment K (blood temperature); *a*, wrapped; *b*, unwrapped; *c*, wrapped; *d*, unwrapped, fan on back; *sh*, shivering begins; *e*, fan off, wrapped; shivering stops; *f*, unwrapped, cold, wet towel to back; *sh*, is shivering; *g*, dried and wrapped; *h*, cold, wet towels to feet, fan on feet; *p*, more cold water poured on towels around feet; *r*, respiration is exaggerated; *q*, fan off, feet dried and wrapped.

ably two factors play a part in the sudden rise in temperature produced; the momentarily increased pressure between terminals and mucous membrane slightly increased, mechanically, the temperature of the former; the painful mechanical irritation of the mucosa by the metal terminals and applicator probably caused a transient blush. The rises in temperature, which took place

before chilling, although apparently elicited by less movement, were much more marked than that which occurred during the active vasoconstriction with chilling— $1.77^{\circ}$  and  $2.69^{\circ}$  as compared with  $.98^{\circ}$  C.

The effect upon skin temperature of inhaling amyl nitrite is shown (2:17). A rise of  $.50^{\circ}$  C. is again produced.

It is to be remembered that, since the mucous membrane and skin curves of each experiment are synchronous, the annotations, although drawn in as though applying only to one curve, apply equally to both.

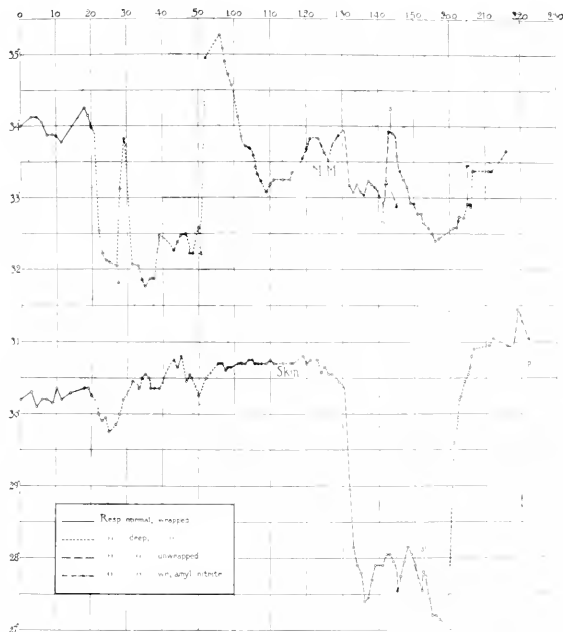


Fig. 6.—Effects of chilling, mechanical irritation and amyl nitrite. Experiment B (temperatures of skin and mucous membrane of oropharynx): *a*, coughs and clears the throat; *b*, clears throat and swallows; *c*, swallows; *d*, coughs and clears throat; *e*, begins shivering; *f*, misses several respirations; *g*, exact time of point *p* not known.

EXPERIMENT C.—M.M. thermopile on posterior wall of *nasopharynx*. Skin applicator on forehead. At first, nostrils plugged, breathing through mouth; later, breathing through nose, mouth open. Respiration, 18 per minute. Thoracic and abdominal pneumographs. Room temperature,  $15.7^{\circ}$  to  $16.9^{\circ}$  C.; time, 4:15 to 5:45 P.M.

With the anterior nares plugged and the soft palate raised in mouth breathing, the nasal chamber was virtually a closed cavity, whose temperature did not vary with the rate of blood flow through its walls. Correspondingly, the mucous membrane curve (Fig. 7) showed no certain change when, at 0:08.5, respiration was deepened, nor at 0:22, when the subject was unwrapped

and chilled with the electric fan. The skin curve, on the other hand, dropped off characteristically, to recover when the subject was rewrapped.

At 0:46 the nostrils were unplugged and nose-breathing began. The nasopharynx at once came into free communication with the outer air, and was cooled by each respiration, and became dependent upon its blood supply for maintenance of its temperature. When equilibrium had been reached, the subject was again, at 1:07.5, unwrapped and chilled with the fan. Mucous membrane and skin temperatures fell together, the former reaching a maximum depression of  $1.83^{\circ}\text{C}$ . in six minutes, the latter of  $1.95^{\circ}$  in eight minutes. After

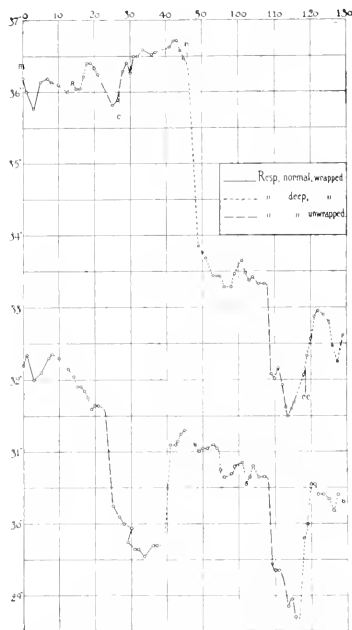


Fig. 7.—Chilling effect. Experiment C (temperatures of skin and mucous membranes of nasopharynx): *m*, nostrils plugged, breathing through mouth; *c*, coughs; *n*, plugs removed from nostrils, breathes through nose; *cc*, coughs three times.

rewrapping at 1:16, the mucous membrane temperature rose in six minutes to a maximum point  $.38^{\circ}$  below the control level, then fell slightly; the skin temperature mounted in four minutes to a maximum only  $.10^{\circ}$  below control level.

In the experiments of the first series a single exception to the rule that mucous membrane temperature, upon wrapping after chilling, does not regain its original level, was found in the experiment performed upon a faucial tonsil. In this case mucous membrane temperature rose well above control level. With this idea of determining whether or not this behavior was characteristic, and because of the peculiar importance of the tonsils as a site of infection, a second

series of experiments was performed with especial reference to the tonsillar reactions. These confirmed the earlier observation.

The curves on the right of Fig. 8 are a composite of four experiments upon tonsil and skin; on the left are shown for comparison composites of two experiments with the soft palate and one with the oropharynx as mucous membrane site of application.

In the tonsillar curves chilling begins at 0:15; mucous membrane and skin temperature fall away steeply together. Upon rewarming at 0:27 skin temperature returns not quite to control level, tonsillar temperature considerably above it. In the palatine-pharyngeal curve, on the other hand, the character-

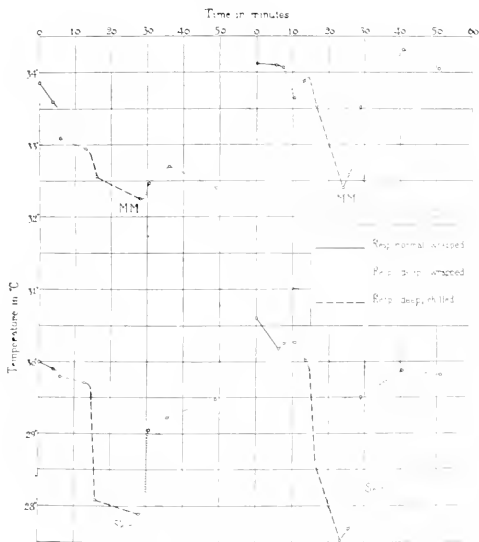


Fig. 8.—Reactions to chilling and to rewarming. On left, composite of three experiments. (Temperatures of skin and mucous membrane of soft palate and oropharynx.) On right, composite of four experiments. (Temperatures of skin and mucous membrane of faucial tonsils.)

istic failure to regain normal relaxation of vasomotor tone after chilling is again evinced.

EXPERIMENT D.—M.M. thermopile on left *faucial tonsil*. Skin application on forehead. Nostrils plugged, breathing through mouth. Respiration, 14 per minute. Thoracic and abdominal pneumographs. Room temperature 17.3° to 19.45° C.; time, 11 A.M. to 12 M.

Experiment D illustrates the same reactions as the composite. With the beginning of chilling at 0:12 skin and mucosa lose heat together, to regain it on rewarming at 0:17.5. By 0:34.5 the mucous membrane temperature had risen to 33.85° C., 1.32° above the level at which chilling began. At 0:35 an ampule of amyl nitrite is inhaled. The vasodilation in the already hyperemic

tonsillar mucous membrane was evidently insufficient to counterbalance the effect of lowered general blood pressure, so that a fall in temperature resulted. The skin temperature on the other hand, rose characteristically with the amyl nitrite. As the flush passed, skin temperature fell and mucosal temperature rose to its former level.

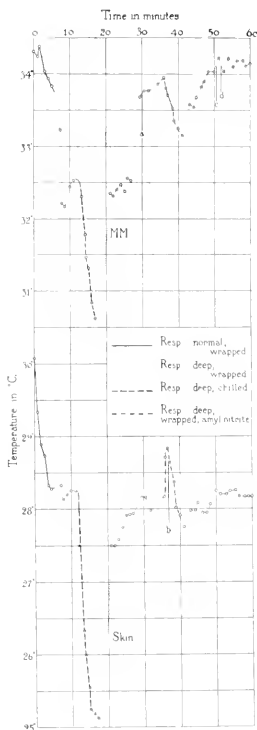


Fig. 9.—Chilling, recovery and amyl nitrite reactions. Experiment D (temperatures of skin and mucous membrane of faucial tonsil): *a*, applicator seen to be all right; *b*, flush fading; *c*, respiration shallow; *d*, breathes deeper.

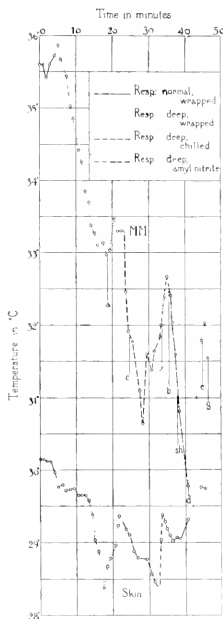


Fig. 10.—Chilling and amyl nitrite reactions. Experiment E (temperatures of skin and mucous membrane of faucial tonsil): *a*, clears throat; *c*, coughed; *f*, face flushed; *b*, flush passing, amyl nitrite taken away; *sh*, shivering; *d*, clears throat; *e*, respiration too shallow, deepened; *g*, subject fainted.

EXPERIMENT E.—M.M. thermopile on left *faucial tonsil*. Skin applicator on forehead. Nostrils plugged; mouth breathing. Respiration 14 per minute. Thoracic and abdominal pneumographs. Room temperature 17.5° to 18.55° C.; time, 10:56 to 11:43 A.M.

Fig. 10 with the beginning of chilling at 0:23 shows the usual temperature fall; the recovery curve was terminated prematurely by the subject unfortunately fainting. Amyl nitrite was administered during chilling at 0:32.5. Vaso-



dilation here evidently more than counteracted blood pressure fall, for the skin and mucous membrane temperatures each rose steeply.

The reactions of the *nasal cavity* have recently been found to be similar in quality to those of the oropharynx and nasopharynx, but quantitatively much more striking. Twelve experiments have been performed on seven different subjects of Aryan, Semitic and Mongolian stock. The sites tested are the nasal septum, inferior and middle turbinates, and inferior and middle meati, all, because of difficulty in application farther back, in the anterior half of the nasal cavity.

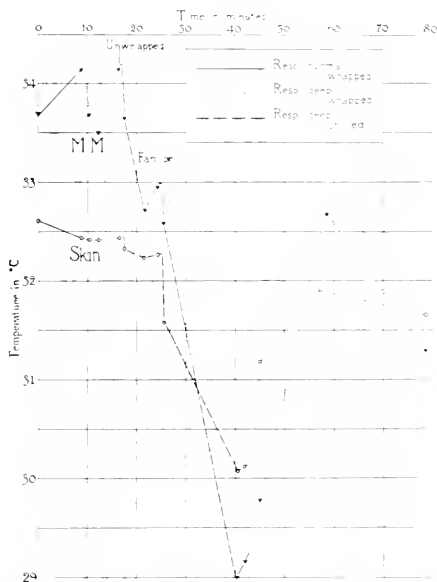


Fig. 11.—Reactions of nasal cavity to chilling and rewarming of body surface. Composite graph of seven experiments. (Temperatures of skin and of mucous membrane of nasal septum, inferior turbinates and middle meatus, in anterior half of nasal cavity.)

In every case chilling of the body surface has resulted in reflex vasoconstriction in the nasal mucous membrane, rewarming in vasodilation. Striking vasodilation occurs on inhaling amyl nitrite.

The reflex to the nasal mucous membrane, as is also the case for the mucous membranes of the palate, nasopharynx, and oropharynx, and for the skin of the base of the neck, appears to have a lower threshold than the corresponding reflex to the skin of the forehead. Merely unwrapping the subject in a room of  $14.0^{\circ}$  to  $18.0^{\circ}$  C. in a number of instances resulted in no change or a very slight fall in skin temperature and a fall in mucous membrane temperature of  $1.5^{\circ}$  to  $2.0^{\circ}$  C., or even more.

Application to the nasal mucosa of the thermopile terminals was extremely irritating and resulted in discharge of considerable amounts of clear mucus. The discharge was both on the side of the cavity in which application was made and on the opposite side, although more abundant on the former. It seemed to be little if at all affected by the marked shrinkage of the mucous membrane during chilling. In three experiments the subject thought secretion was slightly more copious during chilling; in one he thought it slightly less. In others no change in secretion was noted. These last observations bring out two facts:

(1) Discharge from the nasal cavity may be reflex and may occur in regions of the nose not directly irritated.

(2) Rhinorrhea is not necessarily accompanied by vasomotor turgescence in the nasal cavity.

The nasal vasomotor reactions are illustrated by Figs. 11 and 12.

Fig. 11 is a composite of seven experiments of similar pattern performed upon four subjects. The sites used were the right and left sides of the nasal septum, the right and left inferior turbinates, and the left middle meatus.

A transitory fall of  $0.6^{\circ}$  C. in mucous membrane temperature followed deepened respiration. On unwrapping the subject, mucous membrane temperature was depressed  $1.4^{\circ}$  C., skin temperature synchronously only  $0.2^{\circ}$  C. Turning an electric fan on the subject's lower back resulted in a further mucous membrane fall of  $3.7^{\circ}$  C. and a skin fall of  $2.1^{\circ}$  C. Maximum recovery for mucous membrane was  $3.7^{\circ}$  C. (71 per cent); for skin the corresponding recovery was  $1.8^{\circ}$  C. (77 per cent).

EXPERIMENT F.—M.M. thermopile on anterior end of *left inferior turbinate*. Skin applicator on forehead. Mouth closed; nasal breathing. Respiration 11 per minute. Thoracic and abdominal pneumographs. Room temperature  $16.0^{\circ}$  to  $17.0^{\circ}$  C.; time, 1:14 to 2:48 P.M.

Fig. 12 illustrates in an individual experiment the reactions brought out in the composite. With unwrapping at :12.0 the skin temperature is not depressed for two and a half minutes; the mucous membrane temperature in like time falls  $1.6^{\circ}$  C. The pronounced drop in both mucous membrane and skin curves with fan on is interrupted by a sharp rise following amyl nitrite administration at :23.25, amounting, in the case of the mucosa, to  $3.9^{\circ}$  C., in that of the skin to  $1.1^{\circ}$  C. After unwrapping, the mucous membrane temperature in this experiment slightly more than regained its level of before chilling,—(in most of the experiments it remained depressed). Inhalation of amyl nitrite in this flushed condition of the mucous membrane resulted in a momentary depression of  $0.2^{\circ}$  C., followed by a rise of  $0.6^{\circ}$  C. Skin temperature rose  $1.0^{\circ}$  C., approximately as before. The experiment ends with a profound vasoconstriction of mucous membrane and skin vessels incident on a second chilling with the fan.

Checking of quantitative results by *qualitative observations of redness*: The experimental analysis outlined above adequately proves, it seems to us, that chilling of the body surface reflexly produces vasoconstriction in the vessels supplying the normal mucous membranes of the palate, tonsils, oropharynx and nasopharynx, and of the nasal cavity. However, in order to secure still further corroboration, observations of the appearance of the mucous membranes and their blood vessels were made.

Twelve experiments were performed. Of the five observers who noted the appearance of the buccal and oropharyngeal membranes before and after direct exposure of those membranes to cold air, all were of the opinion that, with the direct chilling, blanching occurred. Of the six observers who noted the

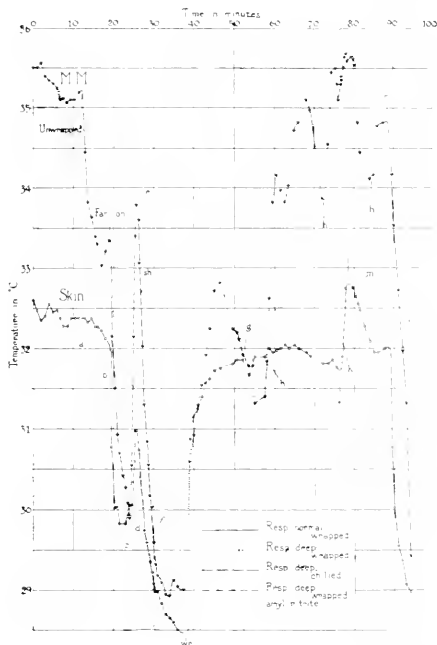


Fig. 12.—Chilling, rewarming and amyl nitrite reactions. Experiment F (temperatures of skin and mucous membrane of left inferior turbinate, anterior end): *a*, subject unwrapped; *b*, fan on; *c*, amyl nitrite inhalation begins; *d*, face has begun to flush; *e*, flush fading, stops inhaling; *sh*, shivers; *f*, is shivering; *wr*, fan off, warmly wrapped; *g*, *m. m.* applicator feels to subject to be in correct position; *h*, *m. m.* applicator readjusted so that subject can feel pressing against turbinate; *j*, amyl nitrite administered; *k*, face flushed; *l*, stops inhaling amyl nitrite; *m*, flush fading; *n*, unwrapped, fan; *o*, is shivering hard.

appearance of the soft palate and the seven who studied the normal oropharynx before and after chilling of the body surface, all said that the membranes paled while the skin was being chilled. Only one observer specifically studied the reaction of the tonsils. He said that they blanched during the skin's chilling. Of the three observers who watched the changes following rewarming, one simply said that reddening occurred; two said that reddening occurred only to a slight extent.

The first, i. e., the response of the mucous membranes to direct chilling, was not suitable to thermogalvanometric study. The observation that blanching occurred is, however, in harmony with observations upon direct cooling of the nasal mucous membrane made by Cocks (1915). The other points observed are wholly in accord with the quantitative studies.

Results in harmony with our own have been published by Tschalusow (1913) who used the nose with posterior nares packed, as a plethysmographic chamber and studied the results of chilling the feet and lower legs and of other sensory stimulation, and by Galeotti (1920) who studied the effect upon the temperature of the expired air of cutaneous chilling and warming. Tschalusow found that in a human subject emersion of the feet and lower legs in water at 18° C. caused a marked decrease in the volume of the nasal cavity; electrical stimulation, needle pricks and scratching the skin caused vasoconstriction also, but to a less degree. Galeotti (1914) found that the temperature of the expired air is depressed 1° to 2° C. by cutaneous chilling, raised correspondingly by warming. In explanation of this he advanced the somewhat improbable hypothesis of pulmonary vasoconstriction.

Vasomotor reactions of abnormal tissues.—Reactions in chronic inflammatory throats: One new point of great interest was brought out by the observation experiments in which the pillars and tonsils were seen to blanch with the cutaneous chilling. The pharynx of this same subject was much injected. He gave a history of having noticed a sore throat about a week before, which he thought had cleared up; the night before the experiment he had driven an open machine without an overcoat, and his sore throat had returned. The diagnosis was chronic catarrhal pharyngitis with pustules. This inflamed throat did not pale with the rest of the mucous membrane, but if anything, its injection was intensified by chilling the body surface.

In a thermogalvanometric experiment upon a case of chronic pharyngitis of two years' standing, a similar reaction resulted (Fig. 13).

*History.*—Began smoking 1915; used about four cigarettes daily. In 1916, began smoking fifteen to twenty cigarettes daily; about this time pharyngitis began. Since has smoked eight to ten cigarettes daily. Treated April, May and June, 1917, Johns Hopkins dispensary, with argyrol, etc. No improvement. Treated twice a week with argyrol and silver nitrate, November and December, 1918, in St. Louis. No improvement. Told smoking was probable cause, and if he would stop smoking throat would get well. Condition unchanged August 2, 1918, when experiment was performed.

EXPERIMENT G.—M.M. thermopile on *inflamed posterior wall of oropharynx*. Skin thermopile on forehead. Respiration, 13 per minute. Nostrils plugged; mouth breathing. Thoracic and abdominal pneumographs. Room temperature, 18.7° to 19.5° C.; time, 10:10 to 11:35 P.M.

The skin curve in Fig. 13 is typical. The mucous membrane temperature shows the fall with deepened respiration which of necessity would follow inhalation into the pharynx through the open mouth of increased volume of cold air. However, with chilling, 0:18.5 to 0:42, the mucous membrane temperature, instead of dropping with the skin temperature, shows a slight transient rise. The shape of the curve, reaching its height in the middle of the period of chilling and then slowly sinking, apparently uninfluenced by the cessation of chilling, suggests that it records some slight changes in local vasomotor tone quite independent of the cutaneous chilling, or alterations in general blood pressure, or some slight accidental change in experimental conditions. At all events there is no questioning the fact that the normal reflex vasoconstriction is absent.

Inhalation of an ampule of amyl nitrite (1:08 to 1:09.5) was followed by characteristic vasodilation. Measurement of the respiration record shows breathing to have been suddenly deepened at 1:09 (Fig. 13, *r*) and to have maintained itself abnormally deep until 1:14.5, when the subject could no longer maintain the slow rhythm, and respiration became quick and shallow. The preliminary fall of mucous membrane temperature from 1:09 to 1:10.5 was

doubtless due, therefore, to deepened respiration and a fall in general blood pressure; the rise from 1:10.5 to 1:13.25 was unquestionably due to local vasodilation and occurred in spite of deepened respiration and lowered blood pressure.

Amyl nitrite causes vasodilation by direct action upon the smooth muscle of the blood vessel walls. This typical dilator response of the inflamed throat to amyl nitrite shows, therefore, that the vessel walls are capable of reacting normally. The typical skin curve would indicate proper functioning on the part of the afferent and association elements of the reflex arc. This failure of normal reaction to cutaneous chilling, in so far as conclusions can be drawn from a single experiment, must then be referred to the motor elements of the reflex arc, and probably has its seat in or near the inflamed mucous membrane.\*

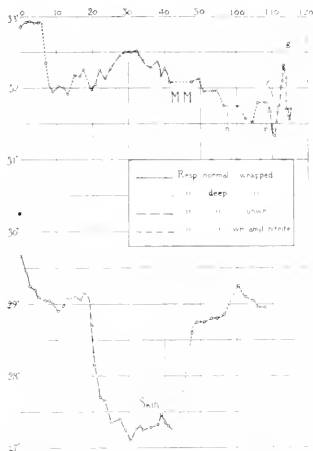


Fig. 13.—Chilling and amyl nitrite effects upon chronically inflamed oropharynx. Experiment G (temperatures of skin and mucous membrane of oropharynx): *u*, plugs pulled from subject's nose; begins nose breathing; *r*, respiration suddenly deepened; *f*, face flushed, *g*, flush passing; respiration still exaggerated.

### Reaction to amyl nitrite of an acute inflammatory palate:

EXPERIMENT H.—M.M. thermopile upon the *intensely injected soft palate*. Acute pharyngitis and tonsillitis. Respiration uncontrolled. Mouth breathing (except 0:00 to 0:23).

At 0:10.5 (Fig. 14) subject inhaled by mouth an ampule of amyl nitrite. A steep fall of mucous membrane temperature amounting to  $2.69^{\circ}$  C. followed. The minimum temperature was reached in 13.5 minutes, and was followed by a rise of  $2.56^{\circ}$  C., attained in four minutes.

Evidently the vessels of the inflamed membrane were practically maximally dilated. The temperature change observed was therefore the result of the increase in depth of respiration and the depression of general blood pressure.

### Reflex reactions of scar tissue to chilling:

\*This experiment is included as suggestive merely, not conclusive; for in later work it was found that a normal throat which ordinarily responded to chilling by reflex vasoconstriction would occasionally fail to exhibit that response. The expiration seemed to be usually or always that excessive swallowing and resultant traumatization of the pharynx occurred during the period of chilling, with consequent dilation of the vessels, as shown in Fig. 6. Such may possibly have been the case in this experiment.

EXPERIMENT I.—Keloid removed from chest wall August 24, 1918. Experiment performed September 25, 1918. Scar at that time 2.5 by 1 cm.; red; covered with epithelium. First thermopile on normal skin of chest near scar. Second thermopile on scar. Room temperature, 21.35° to 21.6° C. (See Fig. 15, curves on left.)

The subject sat wrapped from 0:00 to 0:11.5; the sites of application of the thermopiles were of course freely exposed. At 0:11.5, the subject was bared from the waist up, and chilled with ice bags to her back. Skin and scar temperatures both fell sharply. The scar temperature reached a maximum depression of .90° C. in 1.0 minute, the skin of 1.12° C. in 4.5 minutes. At 0:17.5, the subject gasped and heaved her chest. Possibly as a result of mechanical irritation, the scar temperature at once rose, although skin tem-

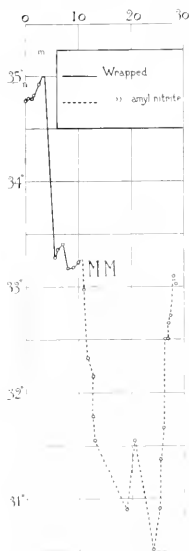


Fig. 14.—Effect of amyl nitrite upon acutely inflamed soft palate. Experiment II (temperatures of mucous membrane of soft palate); *n*, breathing through nose; *m*, changes to mouth breathing.

perature was not affected. At 0:20.75 the subject stood up to wrap herself, and may have disturbed the position of the scar applicator, although no displacement was visible. After wrapping, the skin temperature rose typically, the scar temperature fell.

This experiment on new scar tissue shows, certainly, reflex vasoconstriction in response to chilling. To what degree the later atypical behavior of the scar temperature actually represents its vasomotor reactions and to what the failure of the subject to cooperate, it is difficult to say.

EXPERIMENT J.—Radical operation June 7, 1918, for carcinoma of breast. Experiment performed September 23, 1918. First thermopile on normal skin near scar. Second thermopile breast. Sites of application exposed throughout experiment. Room temperature, 15.65°-16.35° C. (See Fig. 15, middle curves.)

The subject was chilled at 0:07.5 merely by unwrapping in the cold room. Sear temperature dropped  $1.80^{\circ}$  C. within 5.0 minutes; skin temperature,  $2.63^{\circ}$  C. within 5.0 minutes. Upon rewrapping at 0:14.5, sear temperature rose  $.45^{\circ}$  C. in 3.0 minutes; skin temperature,  $1.54^{\circ}$  C. in 4.0 minutes.

EXPERIMENT K.—Admitted to hospital, February 1, 1918, with history of having ulcer on forehead for a year. Diagnosis: syphilitic ulcer. Slow improvement under specific treatment. June 21, 1918, skin graft to ulcer from leg. Favorable. Discharged July 7, 1918. Experiment performed September 12, 1918. Sear about 5 cm. in diameter, red, covered with thin epithelium. First thermopile on normal skin near sear. Second thermopile on sear. Room temperature,  $17.8^{\circ}$  to  $17.95^{\circ}$  C. (See Fig. 15, curves on right.)

At 0:11.5 the subject was bared from the waist up, and an electric fan turned on her back. Hairs over her forehead were not disturbed; thus the sites of application were not much cooled by draft. The sear temperature fell

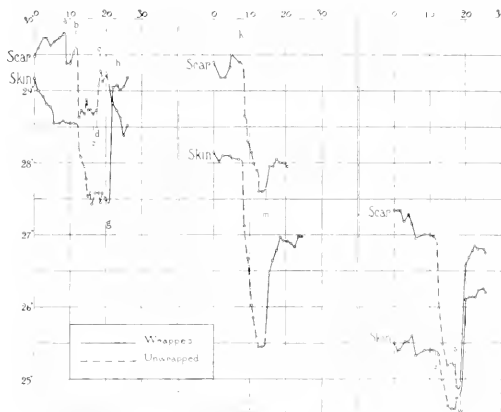


Fig. 15.—Reflex effects of chilling on scar tissue. Experiments I, J, and K (temperatures of scars and contiguous skin): Experiment I: *a*, subject moves, sighs, etc.; *b*, subject bared from waist up; ice bags to back; *c*, gasps; *d*, gasps, heaves chest; *e*, wrapped (subject stood up to wrap self; when she sat down again the skin applicator was found to have tilted; the sear applicator seemed all right, but it also had probably moved somewhat); *f*, heaves sigh. Experiment J: *h*, body bared from waist up; no fan or ice bag used; *m*, wrapped. Experiment K: *n*, body bared from waist up; fan on back; hairs on forehead not disturbed at all by draft; *s*, shivers; *w*, fan off; wrapped.

$2.09^{\circ}$  C. in 6.0 minutes, the skin  $0.82^{\circ}$  in 4.5 minutes. Upon wrapping at 0:18.5, sear temperature rose  $1.96^{\circ}$  C. in 4.0 minutes; skin  $1.55^{\circ}$  C. in 6.5 minutes.

These reactions would seem to explain the cyanosis of fresh sears during cutaneous chilling. The arteriolar constriction doubtless renders the flow of blood through the wide capillary network scant and sluggish, thus allowing unusually complete absorption of oxygen from the oxyhemoglobin.

#### AFTER EFFECTS OF CHILLING-EXPERIMENTS OF SERIES OF 1918

Subject S. B. G., after being chilled as subject for fourteen experiments over a period of twenty-two days in midsummer, developed severe rhinitis, followed quickly by pharyngitis, tonsillitis, and injection of uvula and soft palate. Sub-

ject retained an abnormal susceptibility to colds throughout fall and early winter.

Subject S. M., after being chilled as subject for fifteen experiments over a period of twenty-four days in midsummer, developed a slight neuralgia in left shoulder. Next day was again subject. The day thereafter pleurisy developed in left chest. Neuralgia yielded to local treatment. Pleuritic symptoms lasted two weeks. Mucous membranes remained normal.

Chilled again in November without ill effect. Chilled December 4; experiment 4:00 to 6:00 P.M. By following morning slight congestion in nasopharynx had developed, which persisted three or four days. Chilled January 2 without after-effect other than nausea, vomiting and headache.

This subject noticed that entering the cold room from the hot outside air of summer was frequently followed by violent intestinal peristalsis and defecation. This subject irrigated his nose and throat after each exposure with a weakly alkaline salt solution, used *hot*, with the idea of both cleansing and of producing hyperemia. Following irrigation a spray of 0.5 per cent phenol in liquid albolene was used after the winter exposures.

Subject A. G., on August 1, chilled. After exposure, low grade pharyngitis developed and persisted some days.

Subject J. D. R., with chronic pharyngitis; chilled twice, subject thought after second exposure was exacerbation of pharyngitis.

Subject G. A., suffered after single exposure no ill effects other than headache.

Subject B. J. F., no symptoms after an observation experiment.

#### CONCURRENT BACTERIOLOGIC STUDIES—SUMMER OF 1919

Several instances during the experiments of 1918 seemed at least suggestive of experimental excitation of infection. The case of S. M. in particular seemed to point to chilling as the exciting factor. The experiment was a blood temperature control in which he sat from 4 to 6:09 P.M., December 4, 1918, with closed mouth, the bulb of a thermometer beneath his tongue. He forced respiration from 4:12 to 6:09 P.M., and was chilled by a fan from 4:47 to 5:09 P.M. Shivering began at 4:48 P.M., and had become very severe by 4:52 P.M. The subject had not been aware of other recent exposure to cold or infection. By the morning of December 5, nasopharyngeal stuffiness had developed sufficiently to cause the remark by a friend that he had a cold. The symptoms persisted three or four days.\*

A study of the flora of the nose and throat during the experiments of the summer of 1919 seemed, then, to be well worth while.

*Material and Method.*—The medium employed was a 5 per cent rabbit blood meat infusion agar. Baked blood agar was also used in each instance as a special medium for *Bacillus influenzae*. Sputum from each subject was injected into a mouse for typing pneumococcus. Cultures were taken from the nose through the anterior nares, from the tonsil, and from the posterior pharyngeal wall by means of separate swabs. Each swab was immersed in

\*Similarly we have noted what we believe to be an excitation of infection after, and presumably due to chilling in a number of carefully observed instances in our everyday experience.



sterile broth and then applied both to a red and baked blood agar plate, the remaining area of the plate was inoculated by means of a platinum loop. Films were made directly from the same swab.

The cultures were incubated for 36 hours. The plates were then divided into eight segments, and every colony in two to four segments of the plate was counted and its nature determined. An attempt was made to detect any marked changes in the flora and particularly in the relative proportions of the bacteria present. On account of the difficulty of differentiating pneumococcus from *Streptococcus viridans* by morphology these two were put in the same group.

*Results.*—Four different subjects were used in this study. Two developed clinical sore throats; a third had some symptoms of malaise and headache; a fourth was unaffected. The results obtained with the nose cultures were entirely negative. Subjects A. G. and S. M. showed *Staphylococcus aureus*; subjects W. G. E. and S. B. G., *Staphylococcus albus*. No attempt was made to sterilize the vestibule of the nose before swabbing. The flora obtained from the nose, as has been found by other investigators (Thomson, 1913), was at all times exceedingly sparse, but the above organisms were always found to be present. They showed no changes with exposure. The results obtained from pharynx and tonsil in each instance are given in Tables I to III.

From the pharynx and tonsil of Subject S. M. before the experiment there were cultured *Streptococcus viridans*, *Pneumococcus* Type II atypical, and *Bacillus influenzae*. He was then subject of Experiment 7. Within 24 hours he had a clinical sore throat; coincident with this, there was a sudden appearance of *Streptococcus hemolyticus* in the cultures from both tonsil and pharynx. During the days following, with the disappearance of the sore throat, the number of colonies of *Streptococcus hemolyticus* fell off rapidly. The remaining bacteria showed no evident change.

EXPERIMENT 7.—Subject S. M., June 7, 1919, 3:45 to 6 P.M. Mucous membrane thermometer on left tonsil. Respirations 14 per minute. Mouth open; nostrils plugged. Room temperature 18.10-19.60° C. 0:00 to 0:31.5. Wrapped; normal breathing; swallowed many times. 0:31.5 to 0:44. Wrapped; deep breathing. 0:44 to 0:55.5. Unwrapped; fan on back; deep breathing. 0:52. Shivers. 0:55.5. Coughs, chokes, applicator removed; blood flecks seen about terminals. 4:57 P.M., experiment started again. 0:00 to 0:06. Wrapped; deep breathing. 0:06 to 0:13.5. Unwrapped; fan on; deep breathing; shivering; swallows many times. 0:13.5 to 0:33.5. Wrapped; deep breathing. 0:33.5. Conditions the same; amyl nitrite inhaled. 0:35 to 0:44. Wrapped; deep breathing, some coughing and swallowing. 0:44 to 0:53. Hot water bag around subject; wrapped; deep breathing.

On the following morning the feeling of soreness had practically left the traumatized tonsil, but the posterior wall of the oropharynx felt sore. On inspection a localized area of injection bearing a whitish exudate was seen on the posterior wall of the oropharynx. In culturing, the contact of the swab on this area was a little painful, and the culture yielded streptococci, as explained above. The tonsillar ring was injected, but the feeling of soreness on the tonsil entirely passed off during the day and contact of the swab in culturing was hardly felt. Thus the traumatized tonsil showed less evidence of being the site of an active infection than the posterior pharyngeal wall, which was thought not to have been directly traumatized, but of course we cannot ex-

TABLE I  
SUBJECT S.M. BACTERIOLOGY OF TONSIL AND PHARYNX

DATE	PLACE CULTURED	<i>S. viridans and</i> <i>Pa. II</i>			<i>S. haemolyticus.</i>			<i>B. influenzae</i>			UNDETERMINED		REMARKS
		NO. OF COLONIES COUNTED	PER CENT OF ALL COLONIES	NO. OF COLONIES COUNTED	NO. OF COLONIES COUNTED	PER CENT OF ALL COLONIES	NO. OF COLONIES COUNTED	PER CENT OF ALL COLONIES	NO. OF COLONIES COUNTED	PER CENT OF ALL COLONIES	NO. OF COLONIES COUNTED	PER CENT OF ALL COLONIES	
1919													
June 4	Left tonsil Pharynx	28 36	93 100	0 0	0 0		2 0	7 0	0 0		0 0	0 0	
"	Left tonsil Pharynx	43 31	100 94	0 0	0 0		Present in baked blood				0 4	0 6	
"	Left tonsil Pharynx	35 23	100 86	0 0	0 0		4 4	4 4	0 4		0 4	0 11	
"	Left tonsil Pharynx	52 40	94 87	0 0	0 0		3 4	6 13	0 0		0 0	0 0	Subject of experiment. Applica- tion on left tonsil. Cultures after experiment.
"	Left tonsil Pharynx	57 26	65 56	13 14	15 31		7 6	8 13	11 0		12 0	12 0	Subjective sore throat. Pharynx shows injection with white exu- date; tonsillar ring injected.
"	Left tonsil Pharynx	35 24	76 79	6 9	13 21		1 Present in baked blood	2 blood	4 0		8 0	8 0	Very slight soreness. Throat no longer injected.
"	Left tonsil Pharynx	34 30	94 91	1 3	3 9		1 Present in baked blood	3 blood	0 0		0 0	0 0	Throat normal.

\*Experiment 7.

TABLE 11  
SUBJECT W. G. E. BACTERIOLOGY OF TONSIL AND PHARYNX

DATE	PLACE CULTURED	<i>S. viridans</i>		<i>S. hemolyticus</i>		<i>B. influenzae</i>		<i>S. albus</i>		REMARKS
		NO. OF COLONIES COUNTED	PER CENT OF ALL COLONIES	NO. OF COLONIES COUNTED	PER CENT OF ALL COLONIES	NO. OF COLONIES COUNTED	PER CENT OF ALL COLONIES	NO. OF COLONIES COUNTED	PER CENT OF ALL COLONIES	
1919 June 10	Left tonsil Pharynx	18 13	42 52	4 0	9 0	9 12	21 48	12 0	28 0	
" 11*	Left tonsil Pharynx	28 24	68 72	3 0	8 0	8 6	20 18	2 3	4 9	Subject of experiment. Application on left tonsil. Cultures after experiment.
" 12	Left tonsil Pharynx	31 18	55 53	3 0	5 0	5 12	9 36	17 1	31 9	No after effects.
" 16†	Left tonsil Pharynx	26 32	53 60	0 0	0 0	6 9	12 17	18 12	35 23	Subject of experiment. Application on soft palate. Cultures taken before experiment.
" 17	Left tonsil Pharynx Red blood Eaten blood	14 0 0	45 0 0	2 0 0	7 0 0	8 0 0	26 100 100	7 0 0	22 0 0	Subject complains of general malaise; slight headache; no sore throat. Film from swab shows mostly Gram-negative bacilli with a few cocci.
" 19	Left tonsil Pharynx	19 21	56 60	2 0	6 0	5 14	15 40	8 0	24 0	
" 20	Left tonsil Pharynx	21 19	50 58	3 0	7 0	7 11	17 33	11 3	26 9	

\*Experiment D.  
†Experiment S.

TABLE III  
SUBJECT A.G. BACTERIOLOGY OF TONSIL AND PHARYNX

DATE	PLACE CULTURED	<i>S. viridans</i>			<i>M. catarrhalis</i>			<i>S. aureus</i>			<i>S. albus</i>			REMARKS
		NO. OF COLONIES COUNTED	PER CENT OF ALL COLONIES	NO. OF COLONIES COUNTED	NO. OF COLONIES COUNTED	PER CENT OF ALL COLONIES	NO. OF COLONIES COUNTED	NO. OF COLONIES COUNTED	PER CENT OF ALL COLONIES	NO. OF COLONIES COUNTED	PER CENT OF ALL COLONIES	NO. OF COLONIES COUNTED	PER CENT OF ALL COLONIES	
1919														
June 7	Left tonsil	29	85	0	0	0	0	0	0	5	15			Subject of experiment. Application on left tonsil. Cultures after experiment
"	Pharynx	21	91	0	0	0	0	0	0	2	9			
"	Left tonsil	49	90	0	0	0	0	0	0	5	10			
"	Pharynx	27	75	0	0	0	2	6	6	7	19			No symptoms
"	Left tonsil	21	60	3	9	0	0	0	0	11	31			
"	Pharynx	31	57	15	27	1	2	2	2	7	14			
"	Left tonsil	27	52	4	8	2	4	4	4	19	36			Subject of experiment. Application on right tonsil. Cultures after experiment
"	Pharynx	34	81	0	0	3	7	7	7	5	12			
"	Left tonsil	34	85	1	2	0	0	0	0	5	13			
"	Pharynx	39	93	0	0	0	0	0	0	3	7			Subject of experiment. Application on pharynx. Cultures after experiment
"	Right tonsil	30	73	0	0	4	10	10	10	7	17			
"	Pharynx	34	87	2	5	1	2	2	2	2	5			
"	Right tonsil	40	88	2	5	0	0	0	0	3	7			No cultures. Subject has soreness on swallowing.
"	Pharynx	27	70	7	19	0	0	0	0	4	11			
"	Right tonsil	21	58	12	34	0	0	0	0	3	8			
"	Pharynx	15	45	4	11	11	39			2	5			Subject of experiment. Application on pharynx. Marked congestion of tonsillar ring and pharynx
"	Right tonsil	21	52	11	27	0	0	0	0	8	20			
"	Pharynx	29	69	15	31	0	0	0	0	4	9			
"	Right tonsil	34	72	3	7	8	17	2	2	2	4			Throat injection milder. No soreness on swallowing
"	Pharynx	27	75	6	16	3	8	0	0	0	0			
"	Right tonsil	25	59	2	5	15	36	0	0	0	0			
"	Pharynx	31	77	4	10	5	12	0	0	0	0			Subject of experiment. Application on soft palate. Cultures after experiment
"	Right tonsil	28	54	15	28	9	18	0	0	0	0			
"	Pharynx	32	64	11	22	7	14	0	0	0	0			
"	Right tonsil	35	83	0	0	4	10	3	7	3	7			No cultures. Sore throat disappeared
"	Pharynx	31	93	1	2	0	0	1	3	1	3			
"	Right tonsil	31	81	2	5	1	2	4	11	4	11			
"	Pharynx	27	81	1	3	2	6	3	9	3	9			No soreness. No injection

\*Experiment 9.

†Experiment 6.

‡Experiment 10.

§Experiment 11.

||Experiment 12.

clude the possibility that the oropharynx was infected by hemolytic streptococci from the tonsillar crypts—so commonly a habitat for them—which were missed in the earlier cultures and were disseminated by the experimental trauma and swallowing.

In subject W. G. E. there were present *Streptococcus viridans*, *Bacillus influenzae*, *Streptococcus hemolyticus*, and *Staphylococcus albus*. Following Experiment D there were no noteworthy changes. The plate inoculated from the pharynx about 26 hours after Experiment S, however, showed a pure culture of *Bacillus influenzae*; the tonsillar plate showed also a slight relative increase. The film from the pharynx showed practically all Gram-negative bacilli with an occasional coccus. The subject had no sore throat, but complained of general malaise, slight headache, and some chilly sensations.\* The pharynx culture made 96 hours after Experiment S was practically the same as before the experiment. *Streptococcus viridans* and *Staphylococcus albus* appearing as before.

EXPERIMENT S.<sup>†</sup>—Subject W. G. E., June 16, 1919, 3:20 to 4:07 P.M. Mucous membrane thermopile on anterior half of soft palate, left side. Respirations 16 per minute. Mouth open; nostrils plugged. Room temperature 20.22-20.90° C. 0:00 to 0:05.5, Wrapped; normal breathing. 0:05.5 to 0:11. Wrapped; deep breathing. 0:11 to 0:32. Unwrapped; fan on back; deep breathing. 0:32 to 0:47. Wrapped; deep breathing.

With the applicator resting against the anterior soft palate there is no reason for supposing trauma to the tonsils and pharynx. Organisms could hardly have been introduced from outside by the applicator, for this, with the thermopile terminals attached, was freshly coated with shellac in alcoholic solution before being adjusted for an experiment.

Subject A. G. was the subject of five experiments, June 8, 10, 13, 16, and 18. On June 15 he noted a soreness on swallowing, and on June 16 the entire posterior pharynx and tonsillar ring were distinctly injected. The experiment of June 16 produced no sudden increase in symptoms; on June 17 the sore throat had practically disappeared. On June 19, 36 hours following an experiment, the subject again developed a soreness on swallowing, with congestion of the posterior pharynx and tonsillar ring. On June 20 the symptoms had subsided. On June 21 there was no longer any injection or soreness.

This subject had present in his throat *Streptococcus viridans*, *Staphylococcus aureus*, and *Staphylococcus albus*. Twenty-four hours following the first experiment there was noted for the first time the appearance of *Micrococcus catarrhalis*. Subsequently there appeared to be a certain degree of correlation between the appearances of sore throat and the rises in relative numbers of *Micrococcus catarrhalis* colonies. No other change in the bacterial flora was apparent.

EXPERIMENT 9.—Subject A. G., June 8, 1919, 3:38 to 5:06 P.M. Mucous membrane applicator on left tonsil. Respirations 10 per minute. Mouth open; nostrils plugged. Room temperature 18.1-19.45° C. 0:00 to 0:06. Wrapped; normal breathing. 0:06 to 0:15. Wrapped; deep breathing. 0:15 to 0:43. Unwrapped; fan on back; deep breathing; some swallowing, coughing, and clearing of throat; after 0:17, shivering. 0:43 to 1:20. Wrapped; deep respiration. 1:07. Inhales amyl nitrite. 1:20 to 1:28. Hot water pad to back; deep respiration.

The possibility that trauma to the tonsil was responsible for the appearance of *Micrococcus catarrhalis* after this experiment cannot be excluded.

\*Compare the effect of infecting monkeys with *B. influenzae* (Blake and Cecil, 1920).

†For Experiment D, see p. 259.

EXPERIMENT 6.—Subject A. G., June 10, 1919, 11:30 A.M. to 12:17 P.M. Mucous membrane thermopile on right tonsil. Skin thermopile on forehead. Respirations 16 per minute. Mouth open; nostrils plugged. Room temperature 16.9°-17.8° C. 0:00 to 0:06.5. Wrapped; normal breathing. 0:06.5 to 0:18.5. Wrapped; deep breathing. 0:18.5 to 0:32. Unwrapped; fan on back; deep breathing; after 0:23.5. Shivering. 0:32 to 0:47. Wrapped; deep breathing; contraction of pharyngeal muscles.

EXPERIMENT 10.—Subject A. G., June 13, 1919, 3 to 3:46 P.M. Mucous membrane applicator on posterior wall of oropharynx. Respirations 16 per minute. Mouth open; nostrils plugged. Room temperature 18.45-19.05° C. 0:00 to 0:04.5. Wrapped; normal breathing. 0:04.5 to 0:15. Wrapped; deep breathing. 0:15 to 0:26. Unwrapped; fan on back; deep breathing; coughed; cleared throat; contraction of pharyngeal muscles; after 0:22, shivers. 0:26 to 0:46. Wrapped; deep breathing; coughed and cleared throat several times; pharynx appeared normal.

EXPERIMENT 11.—Subject A. G., June 16, 1919, 10:30 to 11:30 A.M. Mucous membrane applicator on posterior pharyngeal wall. Respirations 14 per minute. Mouth open; nostrils plugged. Room temperature 18.90°-19.80° C. 0:00 to 0:04.5. Wrapped; normal breathing; contractions of pharyngeal muscles. 0:04.5 to 0:23.5. Wrapped; deep breathing. 0:23.5 to 0:32. Unwrapped; fan on back; deep breathing. 0:32 to 1:00. Wrapped; deep breathing; contractions of pharyngeal muscles.

EXPERIMENT 12.—Subject A. G., June 18, 1919, 10:20 to 11:12 A.M. Mucous membrane applicator on soft palate, middle part. Respirations 14 per minute. Mouth open; nostrils plugged. Room temperature, 18.75°-19.55° C. 0:00 to 0:05.5. Wrapped; normal breathing. 0:05.5 to 0:15.5. Wrapped; deep breathing. 0:15.5 to 0:27. Unwrapped; fan on back; deep breathing. 0:27 to 0:52. Wrapped; deep breathing.

In subject S. B. G. there were present in the throat *Streptococcus viridans*, *Pneumococcus* Type IV, and *Staphylococcus albus*. Cultures were made daily from June 4 to 19; he was the subject of an experiment on June 6, 9, 14, and 17. There was practically no change in the bacterial flora of this subject throughout the entire period studied. Neither did subjective or objective signs of sore throat or cold develop. Frequently the cultures from the pharynx were almost sterile, there being from three to ten colonies over the entire plate.

#### DISCUSSION OF BACTERIOLOGIC RESULTS

*Streptococcus viridans* was found in all four of the individuals studied, *Bacillus influenzae* in two, *pneumococcus* in two, and *Micrococcus catarrhalis* in one subject.

In subject S. M. the increased number of *Streptococcus hemolyticus* was definitely synchronous with the presence of a sore throat. There appears to have been a correlation between the high *Micrococcus catarrhalis* counts and the presence of sore throats in Subject A. G. The pure culture of *Bacillus influenzae* in the pharynx of Subject W. G. E. was not coincident with sore throat, but with malaise, slight chilliness, and headache.

These results in no sense prove, however, that the sore throats were caused by the increased number of bacteria cultured from the mucous membranes, or that the apparent increase of microorganisms was caused by the ischemia of the mucous membranes incident upon chilling of the body surface. The method is subject to so many sources of error, and the amount of data thus far obtained is so small, that we do not feel justified in drawing any conclusions. To attribute the apparent proliferation of pathogenic microorganisms to the effect of chilling would seem to be in harmony with the great wealth of clinical and

common observation which points to excessive chilling, under proper circumstances, as an efficient excitant of infection of the mucous membranes by their indigenous pathogenic bacteria. Although it is possible that the apparent proliferation was due to the local ischemia incident upon chilling, the inaccuracy of the bacteriologic method and the insufficient data make it impossible to assume that this is so. The effect of trauma by the thermopiles, the possibility of transient changes in the flora of the mucous membranes caused by swallowing, gagging, or other muscular activity in the pharynx pressing a plug of bacteria from the tonsillar crypts, the fact that the subject's mouth was held open throughout the experiments, with the accompanying accumulation of mucus on the membranes, the errors necessarily introduced in each stage of making the cultures, and the inaccuracy of any method depending upon swab cultures, all tend to confuse the results. We present the data given above as a contribution to the etiology of upper respiratory infections, and not with the idea that the study is in any sense complete in itself.

# LABORATORY METHODS

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## REPORT ON FIVE THOUSAND BLOODS TYPED USING MOSS'S GROUPING\*

BY WILLIAM L. CULPEPPER, B.Sc., DR.P.H., M.D., AND MARJORIE ABLESON,  
DETROIT, MICH.

THERE is at present one of two methods usually employed in typing blood, namely, that of testing the recipient's blood against the donor's *directly* and the *grouping* method. The second method mentioned was the one used in this series of five thousand tests. Until 1901 when a blood transfusion was carried out there were no blood tests made of donor or recipient for hemolysis or agglutination. A number of cases of transfusion with disastrous reactions were reported. This was responsible for so few transfusions prior to that date.

Landsteiner (Ueber Agglutinationsercheinungen Normalen Menschlichen Blutes, in *Wiener Klinische Wochenschrift*, 1910, xiv, 1132) classified all human sera into three groups claiming that all human serum when typed would fall into one of these groups. He designated his groups as follows: Groups A, B, and C.

### GROUP A

- Group A Serum agglutinates Corpuscles of Group B.
- Group A Corpuscles agglutinated by Serum of Groups B and C.

### GROUP B

- Group B Serum agglutinates Corpuscles of Group A.
- Group B Corpuscles agglutinated by Serum of Groups A and C.

### GROUP C

- Group C Serum agglutinates Corpuscles of Groups A and B.
- Group C Corpuscles not agglutinated by Serum of Groups A and B.

This grouping was accepted by the profession until 1910 when Moss began his investigation. In 1907, Jan Jansky (in the review of a paper entitled "Haematologische Studien bei psychotikern," Klineky Sbornik. No. 2, 1907, in *Jahresbericht für Neurologie und Psychiatric*) classified human sera into four groups. In 1910, W. L. Moss (Johns Hopkins Hospital Bulletin, 1910, xxi, 63) pointed out the fallacy in Landsteiner's grouping, which can be seen if compared with Jansky's or Moss's and he (Moss) reclassified them making four groups. Moss's grouping is as follows:

### GROUP I

- Group I Sera agglutinate no corpuscles.
- Group I Corpuscles agglutinated by sera of Groups II, III and IV.

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\*From the Research Laboratory of Parke, Davis & Co., Detroit, Mich.



## GROUP II

Group II Sera agglutinate corpuscles of Groups I and III.

Group II Corpuscles agglutinated by Sera of Groups III and IV.

## GROUP III

Group III Sera agglutinate corpuscles of Groups I and II.

Group III Corpuscles agglutinated by Sera of Groups II and IV.

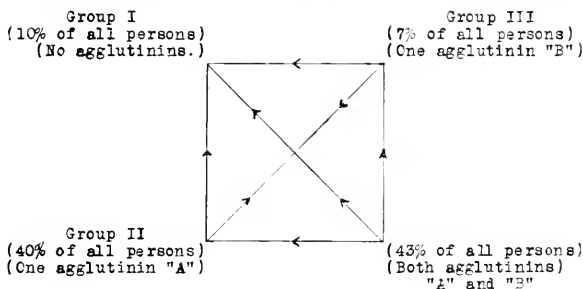
## GROUP IV

Group IV Sera agglutinate corpuscles of Groups I, II, and III.

Group IV Corpuscles agglutinated by no serum.

The only difference between Jansky's and Moss's grouping, is Jansky's Group I corresponds to Moss's Group IV and vice versa. This grouping is being followed at the present time and forms the basis of this report.

As will be noted, Group I contains no isoagglutinins or isohemolysins. Group II contains one agglutinin designated "A." Group III contains one agglutinin designated "B." Group IV contains two agglutinins "A" and "B."



Moss's investigations based on 1600 tests showed that 10 per cent of all persons belong to Group I, 40 per cent to Group II, 7 per cent to Group III and 43 per cent to Group IV.

Our investigations, based on 5,000 tests are recorded in this preliminary report. The ultimate plan being to type 10,000 or more sera. We obtained somewhat different results when compared to Moss's investigations. The sera used were from adults of all ages, from all classes of society, white and negro races. Of the 5,000 examined, 256, or 5.18 per cent were found to belong to Type I; 1805, or 35.06 per cent, to Type II, 714, or 14.28 per cent, to Type III; and 2,225, or 44.48 per cent, to type IV.

The technic of the test is as follows: Two drops of blood containing known Type II corpuscles were suspended in 1 c.c. of a 1.5 per cent solution sodium citrate in 0.9 per cent sodium chloride. A like suspension of Type III corpuscles was also made. A clean sterile glass slide was marked with the name or number of the serum to be typed. With a small sterile pipette two drops of unknown serum to be tested were placed on the slide, one on the left and one on the right. With another sterile pipette, one drop of the suspension of Group II corpuscles was mixed with the serum on the left. With another sterile pipette one drop of

## MOSS'S TABLE

First 1,600 tested = 10% Group I, 40% Group II, 7% Group III, 43% Group IV

## OUR TABLE

First	1000 tested = 3.2% Group I, 36.0% Group II, 11.7% Group III, 47.1% Group IV
Second	1000 tested = 5.0% Group I, 36.3% Group II, 13.5% Group III, 45.2% Group IV
Third	1000 tested = 4.7% Group I, 35.2% Group II, 15.6% Group III, 44.5% Group IV
Fourth	1000 tested = 5.5% Group I, 36.5% Group II, 15.0% Group III, 43.0% Group IV
Fifth	1000 tested = 5.5% Group I, 36.3% Group II, 15.6% Group III, 42.6% Group IV
<hr/>	
Total	5000
	25.9% 180.3% 71.4% 222.4%
	5 / 25.9% Group I, 180.3% Group II, 71.4% Group III, 222.4% Group IV
Average per 1000	5.18% 36.06% 14.28% 41.48%

the Group III suspension was mixed with the serum on the right, every precaution being taken to avoid mixing corpuscles of Group II with Group III. Observations were taken every 3 minutes for one-half hour until reaction was complete. Reactions were interpreted as follows:

1. If no agglutination takes place within 30 minutes the serum belongs to Group I, since Group I serum agglutinates the corpuscles of no other group. Members of this group are called "universal recipients," as they may receive blood transfusions from their own group or any other group. They may be donors for members of their own group only.

2. If the corpuscles of Group III are agglutinated and the corpuscles of Group II remain unchanged, the serum belongs to Group II because Group II serum agglutinates the corpuscles of Groups I and III but not the corpuscles of its own group or those of Group IV. Members of this group may receive blood transfusions from their own group or from Group IV. They may be donors for their own group or Group I.

3. If the corpuscles of Group II are agglutinated and the corpuscles of Group III remain unchanged the serum belongs to Group III because Group III serum agglutinates the corpuscles of Groups I and II but not those of its own group or of Group IV. Members of this group may receive blood transfusions from their own group or from Group IV. They may be used as donors for their own group or to Group I.

4. If the corpuscles of Group II and Group III both show agglutination, the serum belongs to Group IV because Group IV serum agglutinates the corpuscles of all other groups. Members of this group may receive blood transfusions only from members of their own groups. They may be used as donors for Groups I, II, III and IV and are termed "universal donors."

Agglutination and hemolysis may be observed at any time from 60 seconds up to 30 minutes or until the serum evaporates. Hemolysis does not occur without agglutination, but agglutination may occur without hemolysis. Where agglutination only takes place, the corpuscles impart a finely granular appearance to the field. This may usually be seen macroscopically, but can be more readily determined in doubtful cases with the aid of a microscope. Where hemolysis and agglutination both occur the cells are gathered into red clumps or ropes clearly discerned macroscopically. It is possible to determine the group to which an

individual belongs after the slide has dried. Therefore, the slide may be filed away as a permanent record if so desired.

The possibilities that all groups may contain both agglutinins A and B, but in varying amounts or strengths, was suggested by the following observation: A serum giving a marked reaction for a specific group may show a faint inclination to react to the corpuscles of another group, varying from a barely perceptible to a marked reaction. Example—a known suspension of cells of a Group II plus the unknown serum gives a marked agglutination. A known suspension of cells of a Group III plus unknown serum gives a faintly perceptible agglutination. A known suspension of cells of a Group II plus the second unknown serum gives a marked agglutination. A known suspension of cells of a Group III plus the second unknown serum give a moderate agglutination. A known suspension of cells of a Group II plus the third unknown serum gives a marked reaction. A known suspension of cells of Group III plus the third unknown serum gives a strong agglutination. The same suspension of corpuscles were used in this specific observation throughout, it being considered the constant factor.

Second phase of this observation: fresh sera failing to give a positive agglutination for a specific group of corpuscles, when crossed (diluted) with an equal volume of another unknown serum (so far as we were able to ascertain, of the same group by previous typing) and allowed to evaporate at 35° F., approximately 1/10 its original volume, gave a positive reaction. Example—two sera, each showing a positive agglutination for Group III corpuscles and no reaction with Group II corpuscles, macroscopically, were combined in equal volumes and placed in the ice box at a constant temperature of 35° F. for two days, evaporation being encouraged by removal of the stopper from the container. The combined sera were then tested at the expiration of 48 hours with corpuscles (obtained from the same individuals which were used in the first test) of Groups II and III. Both groups gave a positive agglutination, that of Group III being very decided, and that of Group II not so strongly marked but quite unmistakable. This phase would introduce so many possibilities, namely, biochemical changes of the sera or cells, or both, used, that it may be valueless, but the writer thought it well to mention it. On the other hand, it may explain why there is occasionally a reaction, moderate or severe, when transfusions are carried out, by the sodium citrate method, although the donor and recipient belong to the same group.

Third phase of this specific observation: A Group IV serum may contain more isoagglutinins A than B or vice versa. Example—a Group IV serum was diluted with 1.5 per cent sodium citrate in 0.9 per cent sodium chloride. Tests were made with the following dilutions:

- One part serum to one part of the diluent.
- One part serum to three parts of the diluent.
- One part serum to seven parts of the diluent.
- One part serum to fifteen parts of the diluent.
- One part serum to thirty-one parts of the diluent.
- One part serum to sixty-three parts of the diluent.

The result was:

In 1:1 dilution reaction with Group II corpuscles ++++; with Group III corpuscles ++.

In 1:7 dilution reaction with Group II corpuscles ++++; with Group III corpuscles +.

In 1:15 dilution reaction with Group II corpuscles ++; with Group III corpuscles -.

In 1:31 dilution reaction with Group II corpuscles + (with microscope) with Group III corpuscles -.

In 1:63 dilution reaction with Group II corpuscles -+ (partial with microscope) with Group III corpuscles -.

This phase of the investigation was repeated several other times with other sera of Group IV and each time the results checked, that is, the reaction never disappeared from both when using the same dilution.

Fourth phase: The possibilities are that Group I may contain isoagglutinins A, B or both, but in such minute amounts that the reaction, if there is one it is not appreciable.

#### GROUP IV SERUM

SERUM USED	DILUENT	PROPORTION	USED CORPUSCLES OF GROUP	REACTION	USED CORP. OF GROUP	REACTION
1 part	1 part	1:1	II	Strong	III	Strong
1 "	3 parts	1:3	II	Complete	III	Complete
1 "	7 "	1:7	II	Weak	III	Partial
1 "	15 "	1:15	II	Negative	III	Weak
1 "	31 "	1:31	II	Negative	III	Very faint
1 "	64 "	1:64	II	Negative	III	Negative
1 "	1 part	1:1	II	Strong	III	Strong
1 "	3 parts	1:3	II	Strong	III	Strong
1 "	7 "	1:7	II	Weak	III	Weak
1 "	15 "	1:15	II	Negative	III	Partial
1 "	31 "	1:31	II	Negative	III	Negative

#### SERUM IV USED IN FIRST AND SECOND GROUP OF TESTS

SERUM USED	DILUENT	PROPORTION	USED CORPUSCLES OF GROUP	REACTION	USED CORP. OF GROUP	REACTION
1 part	1 part	1:1	II	++++++	III	++++++
1 "	3 parts	1:3	II	+++++	III	+++++
1 "	7 "	1:7	II	++++	III	+
1 "	15 "	1:15	II	+++	III	-
1 "	31 "	1:31	II	++	III	-
1 "	63 "	1:63	II	+	III	-
1 "	127 "	1:127	II	-	III	-

#### SERUM III USED IN SECOND AND THIRD GROUP OF TESTS

SERUM USED	DILUENT	PROPORTION	USED CORPUSCLES OF GROUP	REACTION	USED CORP. OF GROUP	REACTION
1 part	2 parts	1:2	II	++++++	-	-
1 "	5 "	1:5	II	++++++	-	-
1 "	11 "	1:11	II	+++++	-	-
1 "	23 "	1:23	II	++++	-	-
1 "	47 "	1:47	II	+++	-	-
1 "	95 "	1:95	II	++	-	-
1 "	143 "	1:143	II	+	-	-
1 "	179 "	1:179	II	-	-	-

The limitations of dilution of the five thousand sera was fairly constant at a 1:150. Above this dilution we failed to get a reaction. The series were not diluted higher than a 1:9 for grouping the unknown.

Thermal reactions:

First Group of tests.

Group IV serum (undiluted and containing no preservative) tested with corpuscles of Groups II and III, respectively.

At 23° C., reaction strong in both Groups II and III corpuscles.

At 60° C., reaction strong in both Groups II and III corpuscles and much more rapid.

At 65° C., reaction strong in both Groups II and III corpuscles.

At 71° C., serum coagulated making test impossible.

Second Group tests; serum undiluted and containing no preservative.

At 22° C., reaction strong in both Groups II and III corpuscles.

At 60° C., reaction strong in both Groups II and III corpuscles.

At 65° C., reaction strong in both Groups II and III corpuscles.

At 67° C., reaction weak and less rapid.

At 70° C., reaction weak in both Groups II and III corpuscles.

At 72° C., Serum coagulated.

Third group of tests, Type II serum diluted 1:1 and containing 1:250 purified cresols.

At 17° C., strong reaction in Group III corpuscles.

At 60° C., strong reaction in Group III corpuscles and rapid.

At 70° C., strong reaction in Group III.

At 75° C., coagulates.

Fourth group of tests; Type II serum undiluted and containing no preservative.

At 22° C., strong reaction in Group III corpuscles.

At 60° C., strong reaction in Group III corpuscles.

At 71° C., coagulation beginning.

At 72° C., coagulation complete.

Fifth group of tests; Group IV serum undiluted and not preserved.

At 2° C., reaction strong in both Groups II and III but not so rapid as at room temperature.

At 0° C., reaction strong in both Groups II and III but not so rapid as at room temperature.

The serum was then frozen hard, thawed and tested. Reaction was strong and not appreciably retarded.

Influence of preservatives:

A 1:250 purified cresol was used on some Type II and III serum early in March, 1920, and these sera are active on this date. They were kept at ordinary ice box temperature during this time, however. We are now conducting some investigations along this line as to other preservatives.

Influence of syphilis on the reaction:

The table herewith attached represents only sixty-six cases giving a positive Wassermann reaction.

Apparently blood serum that exhibits a positive Wassermann reaction does not change the typing reaction. Moss found this to be the case in 1907.

In typing the number of cases reported here, we selected one thousand sera which had been typed and recorded, then we allowed them to be exposed to the air, which resulted in bacterial contamination. They were then typed again at



Total Group	I = 2	or	3.03%
" "	II = 25	"	37.87%
" "	III = 12	"	18.18%
" "	IV = 27	"	40.90%
Total Groups		= 66	99.98%

the end of six days and we found that they gave the same reaction that they exhibited when first typed. Neither was the time for the reaction to take place altered.

#### CONCLUSIONS

1. Without exception the entire 5000 sera could be grouped and classified according to Moss.

2. Our group percentage in Group I was 4.9 per cent lower than Moss's; in Group II it was 4.1 per cent lower; in Group III it was 7.2 per cent higher; and in Group IV it was 1.5 per cent higher.

3. A permanent slide record may be kept of a reaction if desired.

4. There is a probability that types may overlap. A reaction in the recipient of blood from a donor of the same type may be explained by this probably overlapping.

5. Hemolysis is not specific for any one group.

6. A 1:10 dilution of a serum is very convenient to work with. The maximum dilution we found to be 1:150. The suspension of 1 drop of blood to 1 c.c. of the citrate salt solution is most convenient to work with.

7. The reaction works best at about 40° C. Heat does not impair the reaction until coagulation takes place (at about 71° C). Cold does not interfere with the reaction, although the serum may have been frozen. Bacterial contamination or purified cresol in a dilution of 1:250 does not vary the reaction. Positive Wassermann sera do not influence the typing reaction.

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## EDITORIALS

### *Roentgen Ray Therapy in Hyperthyroidism*

NUMEROUS recent reports of beneficial effects of the roentgen ray and radium in the treatment of hyperthyroidism give evidence of the apparently good results accomplished by the method. While the etiology of the disease remains uncertain, all treatment must perforce be empiric. The malady has been variously ascribed to, (a) psychic influences, such as fear, shock, or other violent emotion; (b) foci of infection, such as tonsils, adenoids, etc.; (c) hyperactivity of other endocrine glands. Possibly the exciting cause differs in different instances, but the predisposing cause is presumably very similar in all cases.

Under medical treatment the symptoms of hyperthyroidism may be relieved, but seldom does the disease entirely disappear within the period of observation. Surgical operation, preceded and followed by prolonged medical care, usually without drugs, has generally been the method of choice. Judd reports the results in a series of 100 consecutive operations done in 1914 and finds that 66 per cent show marked improvement and 5.5 per cent are slightly improved. Eleven of the 100 patients died after leaving the clinic. In a similar series studied in 1909 by the same author, only 44 per cent were



apparently cured. The difference in the two percentages is ascribed to improvements in technique.

The first attempts to treat thyrotoxicosis by the roentgen ray were made as early as 1905. Little has been done however, until during the last five years. Pfahler and Zulick believe that the roentgen ray should be used as a preliminary to surgical operation. They found that by this means the thymus was wholly or partially destroyed and risk from operation was reduced. Kocher had found the thymus enlarged in 50 per cent of his operative cases. The first symptoms of improvement under roentgen ray treatment were found to be increase in weight and decrease in pulse rate. The enlargement of the neck and the exophthalmos did not diminish until late and in many cases did not change.

Means and Aub conclude from their series at the Massachusetts General Hospital that as a rule rest in bed with x-ray treatment should first be followed and continued until the metabolism reaches a level. If this level is not within 20 per cent of the normal it is proper to resort to surgery unless there is a counter indication such as a rising metabolism in spite of complete rest. If the metabolism rises following operation, roentgen therapy should again be applied. Treatment should not be measured by weeks, but by months and often by years.

Hodges believes that early mild or questionably toxic goiters should receive roentgen treatment; that moderately severe cases apparently do better after surgery, and that the severe cases should be irradiated preliminary to the operation. When the result, following operation, is incomplete or unsatisfactory, the roentgen ray will frequently clear up the remaining toxicosis.

Holmes and Merrill feel that roentgen ray treatment accompanied by rest should be tried in all cases and should be continued long enough to destroy at least the thymus before resorting to surgery. They emphasize, however, the importance of a careful diagnosis as to the type of goiter. In simple or colloid goiters and in cystic goiters there is no evidence of hyperplasia of the gland and there are no toxic symptoms. Radiation would only destroy the remaining portion of the normal gland and would hasten hypothyroidism.

These investigators also emphasize the importance of continuing treatment over a long period. In their series, decided improvement was usually seen twelve months after the beginning of treatment. Usually at the end of six months the patients had sufficiently recovered to resume their ordinary occupations, and after one year there was a considerable diminution in the size of the thyroid gland. The results did not appear to be obtained as rapidly as after surgical intervention, but relief appears to have been more permanent, and the danger in the treatment is decidedly diminished.

The dangers of roentgen ray treatment consist in, first, the possible destruction of too great an amount of thyroid substance, with resulting hypothyroidism; second, the possibility of telangiectasis and atrophy in the regions treated. This is particularly to be avoided in young women in whom the resulting disfigurement gives considerable concern. Third, the toxemia of hyperthyroidism may be increased to a dangerous degree by the first

treatment. This may be guarded against by starting with small doses, preceded by rest in bed with medical treatment. Fourth, cases which have had preliminary surgical treatment and are then treated with the x-ray are particularly liable to hypothyroidism and require carefully controlled treatment.

Aiken has used radium in the treatment of toxic goiter and claims equally good results to those following the x-ray. This method also must be reinforced by proper medical care. It is important that the radium be screened so as to prevent the action of the short but powerful beta rays and to obtain the benefit of the more penetrating gamma rays.

The mode of action of the rays has not been clearly worked out. Waters in experimenting on dogs found that the roentgen ray in moderate doses resulted in a cloudy swelling of the thyroid cells. With larger doses coagulation necrosis was produced. It is possible that in a gland showing hyperplasia, these results are even more marked. Hodges suggests that in mild hyperthyroidism, before much if any, hyperplasia has occurred, an induced cloudy swelling may be all that is necessary for successful results.

The x-ray or radium ray will destroy the thymus gland in a short time. Hodges reports that many very large thymus glands under his observation were so reduced within six weeks time that no shadow or widening of the mediastinum could be shown with the fluoroscope.

The method of application according to Holmes and Merrill should be as follows. Treatment should be applied to both the thymus and the thyroid regions. Fairly hard rays should be used and treatment should not be repeated until after a lapse of three weeks. After a series of three treatments there should be an interval of three months, then a second series of three treatments should be given. If the symptoms have not sufficiently disappeared at the end of this period, a third series should be given making nine treatments in all. The patient will be under observation for about one and a half years. This is the technic in general use.

Kingery has recently made a preliminary report in which he suggests another method of therapy. His experience appears to have been chiefly with dermatologic conditions and not with toxic goiters, but the principle involved would appear to be similar in both cases. This author points out that in the older method of roentgen therapy maximum effects were gradually obtained by the frequent administration of small doses over a prolonged period. This is known as the method of "fractional dosage." The method of "massive doses" involves the administration of a maximum dose at one time followed by a prolonged interval without treatment. In either method there is an interval in which the tissue effects are not definitely known. In the first this phase precedes the stage of the erythema that results from cumulative effect. In the second method the uncertain period follows the original maximum effect.

We are accustomed to consider the roentgen ray as being stimulative in small doses, inhibitory in large doses and destructive in very large doses. Kingery points out that in the period of low or partial saturation, preceeding or following maximum effect (erythema dose), there may occur a not inconsiderable amount of stimulation. This, of course, is not to be desired. He be-

believes that the reaction between tissue cells and the radioactive substance follows a law similar to the law of mass action. The greater the concentration of the biochemical products of irradiation, the higher the velocity of their loss. As this concentration decreases, the velocity of loss becomes less in direct ratio. At such a time as this concentration is decreased by one half, the velocity of loss will have become less by a similar amount, and so on until the residual effect has become negligible. This rate of loss theoretically would represent a logarithmic curve.

If such be true, Kingery points out that the curve of residual effect in exposed tissues is logarithmic. The greater the amount of rays absorbed, the higher will be the initial velocity of loss.

From past experience it is known that the residual effect from an erythema dose has become negligible around the fourteenth day after treatment. With this as a basis Kingery constructs a logarithmic curve the ordinates of which express percentage of saturation of the tissues with rays, and the abscissæ the days elapsed since the single massive radiation. With this as a basis he calculates that the tissue saturation at the end of seven days is but 25 per cent while at the end of three and one-half days it is fifty per cent. He proposes now to treat at the end of three and a half days with half of an erythema dose, a procedure which would result in raising the saturation once again to 100 per cent. If treatment is given every three and a half days with 100 per cent saturation the first time and 50 per cent of a full dose on succeeding periods, the saturation of the tissues will at no time fall below 50 per cent. In this way he hopes to eliminate the uncertain period in which stimulation effect may occur. He has found it inadvisable up to the present to decrease intervals to less than half a week.

It is difficult to weigh the relative advantages of the roentgen ray over other methods, in the treatment of toxic goiter. As Holmes and Merrill have pointed out, Hale and White found that out of 87 cases treated medically, with only supportive therapy, 61 ultimately recovered, 21 were decidedly improved and only 5 remained unimproved. Stanton believes that exophthalmic goiter is a self-limiting disease and that 60 to 70 per cent of cases result in spontaneous recovery at the end of five or six years. These figures are very similar to the 66 per cent reported by Judd after surgical treatment.

Hodges found that in his series 50 per cent were apparently cured and 32 per cent greatly benefited by x-ray treatment.

Holmes and Merrill found decided improvement in most of their cases within 12 months after treatment was begun. They recognized that the symptoms had often been present for a considerable time and that possibly the disease had nearly run its course before treatment was begun. Nevertheless they believe that the evidence is sufficient to show that in early cases a more ready response to treatment and more satisfactory results are obtained by roentgen ray therapy.

From a study of these various observations, it would appear that the percentage of recovery by medical, surgical or roentgen ray treatment, individually, is about the same. It may be that following the latter methods recovery is swifter. In many cases without doubt a combination of all three

methods gives the best results. Treatment is most successful when it is individualized. Greater refinements in the technic of radiotherapy and new methods such as the application of Kingery's method to treatment of the thyroid gland may be productive of more satisfactory results. In the mean time it is well to recall that our present methods are but therapeutic patch work as long as we remain ignorant of the pathogenesis of the disease.

—W. T. V.

### *Late Sequelae of Encephalitis Lethargica*

ALTHOUGH "lethargic encephalitis" as such has been described since 1916, attention has been devoted to it particularly during and since the 1918 influenza epidemic. Recently Durand has asserted that the disease is by no means new, that epidemics with quite similar features have been reported from time to time in various countries. A great difficulty in its identification is the large variety of clinical symptoms which may occur in different cases. Epidemiologic considerations aid greatly in the diagnosis. While according to Netter, lethargy and ocular palsies are present in as high as 75 per cent of cases, yet there are many in which the symptoms are complex and simulate other diseases of the central nervous system. This is to be expected with a disease which is apparently infectious and which may localize at various widely separated areas in the brain and spinal cord. Almost any combination of nervous symptoms may occur. Fever is usually present.

Dunn and Heabey have recently described eight clinical groups. It must be borne in mind that the individual members of these various classes all differ somewhat from one another and that the classification is at best artificial. There are cases simulating paralysis agitans and others resembling the condition known as disseminated sclerosis. In the individual case the general features are more valuable in making a diagnosis than is the determination of the portions of the central nervous system involved.

Remond and Lannelongue have followed for a period of ten months, five individuals convalescent from an attack of encephalitis lethargica. Four were men and one was a woman. Some of these had presented paralytic symptoms while others showed clonic manifestations with a remarkable diminution of psychic activity and a marked tendency to somnolence. Two reactions, however, were common to all cases, first a dissociation between the pupillary reaction to light and accommodation, and second an increase above the normal of sugar in the cerebrospinal fluid (.65 to .80 grams.) The pupillary reaction in accommodation was found to be definitely slower than that to light.

These authors were particularly interested in the extreme tenacity and the variety of the pathologic phenomena, and the frequency of relapses. The first patient still complained of symptoms three months after onset of illness; the second, seven months; the third, eight months; the fourth nine months. The fifth, nine months after onset, had been in the hospital for his illness, four times.

Among these five individuals the symptoms were as diverse as they are during the primary attack. Perhaps the most frequent symptoms in the cases were a persistence of the tendency to somnolence and a hyper-irritable condition of the peripheral nervous system. As the authors suggest, the disease is an affection of the nervous system which develops very rapidly and terminates either by death or by recovery, but which in certain cases, becomes prolonged into a chronic affection with symptoms essentially of prostration and hyper-irritability. This irritability sometimes affects the peripheral nerves, sometimes the posterior nerve roots, sometimes apparently the muscles themselves, and sometimes the special senses. Often the late cases resemble multiple sclerosis.

The increase of sugar in the cerebrospinal fluid may last as long as seven months. The authors believe that this indicates a persistence of the infectious agent.

Weber reports the case of a young man who fell a victim to an ambulatory attack of "lethargic encephalitis" in December, 1919. He was sick five months, during which time the predominant symptom was a somnolent condition. He was admitted to two hospitals subsequent to this, the second admission being in September, 1920. In this case the symptom complex resembled paralysis agitans. Janet has also reported an ambulatory case in a child of twelve years which was followed by symptoms resembling paralysis agitans. Similar observations have been made by Kinier Wilson, Eeonomo and by Tretiakoff and Bremer.

It is well in the future to consider "lethargic encephalitis" as an important antecedent in the diagnosis of certain diseases of the central nervous system.

—W. T. V.

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### *Mexican Smallpox*

IN view of our recent editorial, "Are there two diseases included in the present diagnosis of Smallpox", we have received a copy of a most valuable article on smallpox written by Dr. Frank R. Young of Gering, Nebraska. This article was published in the December 1914 number of the Journal of the Arkansas Medical Society. Dr. Young reports in this article a highly fatal epidemic of smallpox which was imported from Mexico. He states:

"Mr. White and family left Tampico, Old Mexico, on December 30, 1913, landed at Port Arthur on the night of January 6, and reached Bert Carson's home in Elm Springs Township on January 8. On January 5, a boy, two and one-half years old, became sick, but his disease was not diagnosed as smallpox until January 10, before which time a number of people had been exposed to the disease. The epidemic spread, finally causing the sickness of twenty-six people. Of these ten died. Of those that died, but one had been vaccinated and he had been vaccinated in 1864. Of the vaccinated people who were in almost constant contact with the patients were Mr. and Mrs. White, two White children and Mr. and Mrs. Phillips. Of these, Mr. and Mrs. White and one White child did not contract the disease. One

White child had the disease in a very mild form, not necessitating going to bed. Mr. and Mrs. Phillips each had the disease in a very mild form. Mr. and Mrs. Phillips were vaccinated in 1864, and no attempt to revaccinate had been made since that time until after this outbreak. The White family were vaccinated in 1910. A number of other persons, who had been vaccinated, came in contact with the disease before its nature was known, and did not contract it. Alex Downum, who had a severe attack of smallpox in the year 1864, at the age of two years, was employed as a nurse in the Phillips home and contracted the disease in a moderately severe form. Other persons, numbering in all about twenty, who had had the disease previously, were employed as nurses and none of them contracted the disease."

This is a very interesting report and we are under obligations to Dr. Young for calling our attention to it. Smallpox of a malignant type was introduced into Mexico at the time of the Conquest by Cortez and his soldiers. This is the Asiatic form of smallpox. It was malignant at that time and has remained so ever since.

—V. C. V.

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### *Fiftieth Annual Meeting of the American Public Health Association*

THE fiftieth annual meeting of the American Public Health Association will be held at New York City in November, 1921. The date which is tentatively announced is November 14-18.

It is interesting to note that Dr. Stephen Smith, the founder and first president of the Association, is now entering his 99th year. He is still active and vigorous and is expected to celebrate his approaching centennial together with the semi-centennial of the Association.

The first organization meeting of the Association was held in New York City on April 18, 1872, and that is one of the reasons for selecting New York City for the celebration of the semi-centennial. Other considerations are the convenience to foreign representatives and to Dr. Smith, who lives in New York City; and especially a plan to conduct demonstrations of public health administrative methods in the laboratories, executive offices, garbage disposal plants, and similar centers of public health interest, in which New York City is unsurpassed.

It is expected to present in connection with the celebration a review of the progress of the various branches of public health within the last fifty years. The sectional programs will include Public Health Administration, Vital Statistics, Laboratory, Food and Drugs, Sociology, Sanitary Engineering, Industrial Hygiene, and Child Hygiene.

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## ORIGINAL ARTICLES

### FACTORS MODIFYING THE DURATION OF VENTRICULAR SYSTOLE\*

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#### I. INTRODUCTION

THE maintenance of an adequate circulation of blood, which is primarily the function of the heart, is accomplished chiefly by the rhythmic activity of the ventricles. Each ventricle operates as a pump, expelling blood by the contraction of its muscles during the phase known as systole and refilling its chamber from the auricle during the phase known as diastole. Although emphasis has been laid on the fact that the circulating minute volume and the systolic and diastolic blood pressures are dependent on the systolic discharge and the rate of the heart, it has not always been appreciated that these must also be considerably affected by the *duration* of the systolic discharge as related to the diastolic time allowed for refilling. The question at once arises as to how much the duration of systole can vary and what factors operate to cause these variations. A critical consideration of the literature and the results of this investigation are presented in this paper in an attempt to answer this question, not only because of its scientific import but also for the practical bearing it may have on the clinical study of the heart.

The heart cycle is divided into systole, or that period during which the ventricular muscle is either mechanically shortening or increasing its tension, and diastole, or that portion of the cardiac cycle between the end of one systole and the beginning of the next. Systole can be further subdivided into an isometric and an ejection period.<sup>20</sup> The isometric period, which begins with the contraction of the ventricle, is so termed because the heart contracts without any change in length. During this period, the ventricle is entirely shut off from the other chambers except for a brief interval at the onset. No blood is therefore expelled from it until the semilunar valves open. The opening of these valves marks the begin-

\*This thesis, awarded the prize offered by the Alpha of Ohio chapter of the Alpha Omega Alpha for 1920, has been somewhat condensed for publication and only the more necessary illustrations have been retained. A detailed report of that part of the work concerning the selective influence of the accelerator nerves has already been published in association with Wiggers in the *American Journal of Physiology*, 1920, liii, 49.

ning of the ejection phase, during which the ventricle contracts tonically and ejects the blood in it into the aorta (or pulmonary conus) with which it then communicates.

Diastole can also be subdivided into a period of rapid diastole, a period of diastasis and a period of auricular activity. The period of rapid diastole begins with the relaxation of the ventricle and lasts a variable time. The ventricle, during the early part of this phase, except for an extremely short interval at the onset, is a closed chamber and no blood enters. Later, however, when the auriculo-ventricular valves open, the ventricle fills with blood from the auricle. The filling of the ventricle continues during the next phase also, but at a much slower rate. This period is termed the period of diastasis as in it the ventricular muscle has reached a stage of rest. Toward the end of this phase another impulse, arising in the sinus node, sets up an auricular contraction, fractionate in character,<sup>26</sup> which constitutes the period of auricular activity. There is still some dispute as to what rôle the auricular activity plays in the filling of the ventricle.<sup>1, 2, 9, 17, 18, 20</sup> The time relation of these various events in the cardiac cycle is well illustrated in Fig. 25 of Wiggers' Monograph.<sup>20</sup>

## II. METHODS OF DETERMINING THE DURATION OF SYSTOLE

The value of the conclusion of any research depends as much on the efficiency of the method employed as on the interpretation of the observations. For this reason a brief survey of the methods available for temporal studies of the cardiac phases is presented.

Any of the changes or combinations of changes that accompany the heart cycle may be utilized to determine the duration of systole. These changes of the heart cycle can be studied in (1) the myocardiograph, (2) the volume curve, (3) the intraventricular, intraauricular and intraaortic pressure curves, (4) the apex beat, (5) the venous pulse, (6) the arterial pulse, both central and peripheral, (7) the electrocardiograph and (8) the heart sounds.

An objection raised to many of these records is that they do not actually measure the entire duration of systole. Thus the myocardiograph, the volume curve and the arterial and venous pulse curves do not record the isometric period, and the results obtained from them must be interpreted as referring only to the ejection period. The electrocardiographic records which have been utilized<sup>25, 30, 50, 53</sup> to determine the duration of ventricular activity are also unreliable for this purpose. The significance of the various deflections are not as yet settled so that the results obtained cannot at the present time be properly interpreted (see Hewlett,<sup>8</sup> Macleod,<sup>12</sup> Wiggers.<sup>20</sup>) Observations which are obtained from graphically recorded curves are also objected to for, due to the short duration of systole, the error introduced in these curves by the recording levers through friction, inertia and momentum is sufficient to lead to erroneous results. Similarly results obtained with some of the membrane manometers, such as Hurthle<sup>40</sup> and Hunt<sup>42</sup> used, are also inaccurate and unreliable (Frank<sup>31</sup>).

For one or other of the above reasons most of the methods mentioned are unsuitable for *accurate* and *cracking* studies of the duration of the heart phases. Those which do not require the opening of the chest, however, can be used for ordinary clinical purposes.



The most accurate and definite estimation of systole can be obtained from a simultaneous tracing of the optically recorded intraventricular and aortic pressure curves, measuring from the beginning of the rise of pressure in the ventricular to the beginning of the incisura of the aortic curve. The objection to such a method lies in the necessity of opening the chest. The circulation under these circumstances is far from normal, due to the shock of the operation as well as the bleeding which occurs even with the best technique.<sup>57</sup> The necessity of deeper anesthesia, the replacement of the negative intrathoracic by atmospheric pressure, the cessation of natural respiration and the utilization of artificial respiration, all aid in making the circulation abnormal.

An attempt was made to select for this investigation that procedure which permits an accurate and exact estimate of the duration of systole and diastole while *still keeping the circulation normal*. The most satisfactory method answering these prerequisites is obtained by recording the heart sounds. Wiggers and Dean<sup>60</sup> have shown that the main vibrations of the first sound, when recorded directly from the heart, begin with the rise of the intraventricular pressure and that the beginning of the second sound coincides with the beginning of the fall of the incisura. These records therefore compare favorably with the simultaneous records of the intraventricular and aortic pressure curves, as to accuracy and definiteness, and have the added advantage of not necessitating the opening of the chest.

Heart sounds have been variously recorded.<sup>7, 22, 41</sup> At the present time there are two generally accepted procedures. In one the heart sounds are registered electrically by means of a microphone connected to a string galvanometer, as is the case in Einthoven's phonocardiograph,<sup>21, 29, 32, 45</sup> The other method records the heart sounds by means of capsules and air transmission.<sup>20</sup> This method has recently been perfected by Wiggers and Dean<sup>60</sup> and has been successfully used to record murmurs and the intensity of the sounds.<sup>58, 59</sup> The clear cut records obtained by this last method were used in this research.

### III. PROCEDURE OF INVESTIGATION

(a) *Animals and Anesthesia*: Dogs, the animals used, were put under the influence of chlorotone and morphine, which gives a lasting anesthesia.

(b) *Operative Procedures*: In addition to the ordinary procedures, namely tracheotomy, vagotomy, the insertion of cannulae into the carotid artery and jugular vein, the stellate ganglion was exposed without resecting any ribs.

The operation performed is very similar to the one first described by Schmeideberg.<sup>4</sup> The animal, during the operation, was kept on a board warmed by electric bulbs. The skin incision, used for the tracheotomy was prolonged to the sternum and then carried at right angles to the end of the shoulder girdle. The skin was next reflected so as to expose the muscular and the subclavian triangles of the neck. The sternomastoid, sternohyoid and sternothyroid muscles were divided close to their insertion into the sternum, pulled back and the vessels beneath them exposed. The pectorales were also divided for half an inch near their sternal end so as to expose the subclavian vessels. The large veins at the root of the neck on one side were dissected out and a ligature tied to each near its entrance into the mediastinum. Another ligature was placed some distance

up and the branches entering between them tied off. The vein between these two ligatures was then resected. The arteries were ligated and removed in a similar manner. In tying the subclavian artery special care was taken not to injure the ansa subclaviae.

After the vessels were tied the previously exposed vagus was dissected free until the branches going to the inferior cervical ganglion were exposed and followed to this ganglion. The esophagus and trachea were then drawn toward the opposite side and by following the branches of this ganglion that proceed posteriorly, medially, and caudad the stellate ganglion, which lies about the junction of the first rib with the vertebral column, posterior to the esophagus, was exposed.<sup>2a</sup>

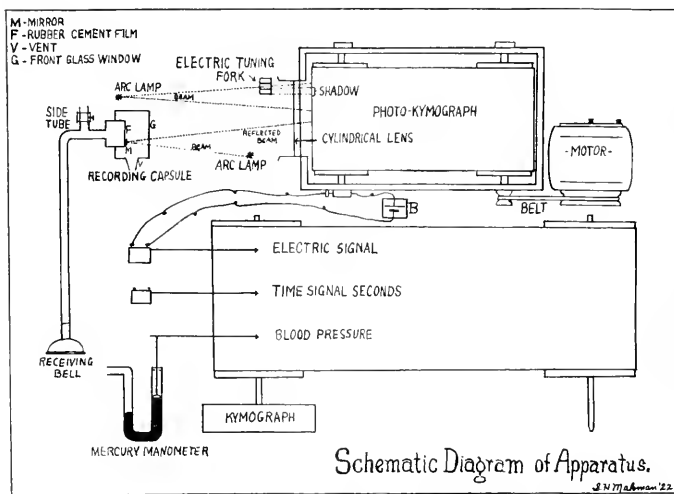


Fig. 1.—Schematic diagram of apparatus used. Description in text.

Some difficulty was occasionally experienced in the last part of the operation until the vertebral vessels, which ordinarily can be pushed aside, were ligated and removed. In working at the root of the neck care was taken not to injure the pleura or the nerves, to tie the ligatures on the vessels tightly so as to prevent hemorrhage or air embolism, and to reduce shock to a minimum. In this way an animal with a practically normal circulation was available.

(c) *Apparatus:* A diagrammatic representation of the apparatus is given in Fig. 1. On a long smoked kymograph, a continuous record of blood pressure was taken by means of a mercury manometer, the time was given by a time-signal recording in seconds. An electric signal was connected to the photokymograph so that it recorded on the smoked drum when and how much of the optical tracing was taken. In addition the signal also served as a base line for the blood pressure. The apparatus for recording the heart sounds is also represented in

Fig. 1. The sounds were picked up from the desired areas by means of a stethoscope bell which was strapped with an elastic belt to the previously shaved chest. The sounds were transmitted to a capsule through a soft rubber tube, which had a side opening to eliminate the cruder variations of pressure, such as the apex beat. The sounds caused the rubber film stretched over the capsule to vibrate. Extraneous sounds were prevented from affecting this membrane by enclosing the capsule in a housing with a window to permit a beam of light through and a vent to equalize the pressures on both sides of the film. A beam of light from an arc lamp was focused on the mirror supported by the film, so that the reflected beam passed through the slot of a photokymograph,<sup>57</sup> which consists essentially of rollers so arranged in a closed box that the bromide paper, rolled up on one of them, moves past a slot and is rolled up on another. To establish time relations the shadow of a tuning fork vibrating fifty times per second was photographed simultaneously with the heart sounds. A typical record thus obtained is shown in Fig. 2.

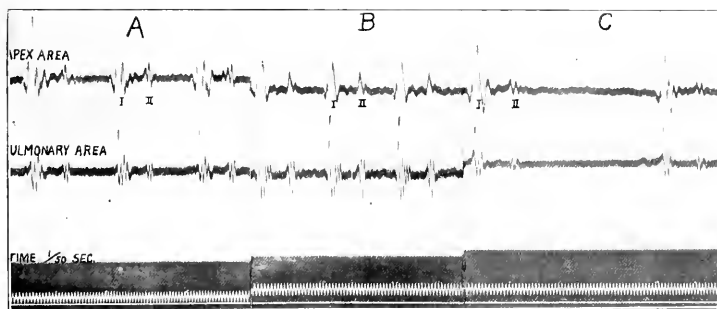


Fig. 2.—Optical records of heart sound (in dog) obtained by direct method ( $\frac{1}{2}$  actual size). A, under normal conditions of anesthesia; B, during stimulation of right stellate ganglion; C, during vagus stimulation. Illustrating the relative alterations of systole and diastole during the action of the two nerves.

(d) *Procedure:* The sounds were recorded during consecutive beats in the following experimental conditions. Vagi intact:

1. During normal action of the heart under anesthesia (called normal standard);
  2. During stimulation of the stellate ganglion;
  3. During action of pituitary extract before, during and after stimulation of the accelerator nerve;
  4. Following section of both vagi.
- After section of the vagi:
5. During stimulation of the peripheral end of the vagi;
  6. During stimulation of the stellate ganglion;
  7. During action of epinephrin;
  8. During the injection of saline intravenously;
  9. During compression of the abdominal aorta.

The records so obtained were developed and the systole and diastole lengths measured. The error introduced in measuring the records is about 0.002 seconds. In all, some three thousand heart cycles were measured and tabulated.

#### IV. A REVIEW OF THE LITERATURE CONCERNING THE INFLUENCES MODIFYING THE DURATION OF SYSTOLE AND DIASTOLE

It is common knowledge that the duration of diastole is very variable, but the fact that systole varies is often lost sight of. The duration of systole may be altered when the duration of either the isometric or the ejection period is changed. This may conceivably be produced by (a) mechanical influences modifying the conditions under which the heart pumps, and (b) nerves, chemicals, etc., presumably altering the physiologic properties of the heart muscle.

(a) *Mechanical Factors*: Theoretically it may be supposed that the duration of the isometric period of systole might change readily. Thus it may be argued that an increase in initial ventricular pressure or a decrease in aortic resistance tends to shorten the isometric period, for it does not take as long for the ventricular pressure to exceed that of the aorta. There is no experimental evidence, however, that variations of the isometric period do play a great part in determining the duration of systole. Thus, Hurlbule,<sup>40</sup> de Heer,<sup>38</sup> Garten<sup>37</sup> and Frank<sup>38</sup> found this period fairly constant and independent of heart rate. More recently Wiggers and Clough<sup>61</sup> found that the isometric period in man varies from 0.04 to 0.06 seconds independent of the duration of the heart cycle or systole. The duration of systole must therefore be largely determined in a mechanical way by influences that effect the duration of the ejection phase. The variations of the ejection phase may be accounted for *a priori* as due to alterations of one or more of three variables: (1) the volume of the ventricle at the beginning of the ejection; (2) the volume at the end of the ejection; (3) the rate of ejection.

The researches of Landois, Donders, Volkman,<sup>7</sup> and later Edgren,<sup>27</sup> Chapman,<sup>24</sup> Porter,<sup>52</sup> Thurston,<sup>54</sup> among others, have led to the commonly accepted conclusion that the main changes with heart rate occur in diastole. That systole varies with heart rate has also been demonstrated [see Curtiss,<sup>3</sup> Foster,<sup>5</sup> Hart,<sup>6</sup> Landois and Sterling,<sup>10</sup> Rollet,<sup>7</sup> Hill,<sup>15</sup> Tigerstedt<sup>19</sup> and Wiggers,<sup>20</sup> (table, p. 41)]. More recently, Wiggers and Clough<sup>61</sup> found that, in man, systole varies from 0.25 to 0.30 seconds for heart rates of 66 to 100, and Lombard and Cope (results as yet unpublished) noted a variation of systole from 0.236 to 0.290 seconds when the heart rate varies from 66 to 89.

Several attempts have been made to correlate the systolic duration with heart rate. Thus, to establish such a correlation Garrod<sup>36</sup> has presented two formulae which he calculated from the cardiogram and radial pulse. For the former he offered the formula  $xy = 20\sqrt{x}$ ; for the latter  $xy = 47\sqrt[3]{x}$ . In both  $x$  is the heart rate per minute,  $y$  the relation of systole to cycle length. Later Lombard and Cope<sup>46, 47</sup> presented the formula  $S = \frac{60}{K\sqrt{R}}$  to determine the length of systole from heart rate,  $S$  being the duration of systole,  $R$  the heart rate per minute and  $K$  a constant varying for position of body, etc. They used the carotid pulse to obtain their data.  $K$ , they found, varied as the patients were lying down, sitting up or standing.

Another attempt to correlate the duration of systole to heart rate may be based on Henderson's volume curves. In 1906, and later in 1909, Henderson<sup>59</sup> promulgated the law of Uniformity of Behavior of the heart. This law maintains that at different heart rates the amplitude but not the contour of the ventricular

volume curve is modified. Henderson found that because the effective venous pressure is normally greater than the critical, the volume curves of the ventricle are superimposable on the curve of a vagus beat (Fig. 3). He further maintained that in accordance with his law, the separate functions of the heart, which Engleman<sup>41</sup> claimed can change independently, vary only with the heart rate. It is evident from his curve that changes in duration of systole with heart rate occur mainly by alteration of the volume of the ventricle at the beginning of systole (Fig. 3). Using his published curve and plotting the temporal relations of systole to cycle (after adding 0.05 seconds for the isometric period) it was found that, until the cycle became less than 0.7 seconds, systole was practically constant. With shortening of the cycle beyond this, however, systole shortened progressively (see Fig. 5).

The heart rate is not the only influence that modifies the duration of systole. Other mechanical influences have been found to do this. Thus, Bowen<sup>22</sup> in 1904 found that systole is prolonged in man at the beginning of exercise before the

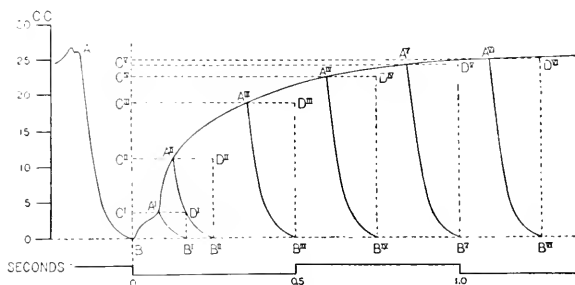


Fig. 3.—Standard volume curve (from Henderson).  $C'D'$  and  $BB'$ , etc., is the duration of the heart cycle;  $C'A'$ , etc., is the duration of diastole;  $A'D'$ , etc., is the duration of the ejection period.

nerves could compensate. Patterson, Piper and Starling<sup>71</sup> in 1914 found that when nerve influences are removed an increase in arterial resistance or in venous return increases the duration of the entire systole. They used their heart-lung preparations to obtain these results. The increase of the diastolic volume of the ventricle, they asserted, is the cause of lengthening of systole. Both the diastolic volume and the volume of the ventricle at the end of systole are altered by changes in arterial resistance or venous flow. They concluded, that in a heart removed from extrinsic nervous influences, these factors could alter the duration of systole independent of the heart rate.

(b) *Influences Other than Mechanical*: Chemicals, as for instance calcium, sodium and potassium ions, have been shown to modify the duration of systole.<sup>19</sup> Temperature likewise modifies the duration of systole as Langendorf<sup>44</sup> has demonstrated.

The cardiac nerves have been thought to have a similar effect. A detailed review of the literature concerning the effect of these nerves on the duration of systole and diastole is given in a recent paper with Wiggers<sup>62</sup> and therefore only a brief summary is presented here. The fact that the vagi and accelerator nerves

modify the heart rate has been known since their discovery by the Weber brothers, Cyon brothers and V. Bering.<sup>13</sup> At the present time it is almost universally accepted that they are the main factors involved. It has also been accepted that the vagi depress all of the heart's functions while the accelerators stimulate them<sup>13</sup> and that these nerves are tonically active,<sup>42</sup> the former being more powerful than the latter.

The work of Baxt,<sup>22</sup> Klug,<sup>43</sup> MacWilliam,<sup>49</sup> V. Frey and Krehl,<sup>55</sup> Hurlthle,<sup>40</sup> Frank,<sup>35</sup> Hunt<sup>42</sup> and Patterson, Piper and Starling,<sup>51</sup> led these investigators to the conclusion that the vagi chiefly affect the duration of diastole while the accelerators mainly alter the duration of systole. Henderson,<sup>39</sup> however, maintained that there is no such difference, the nerves altering the heart rate primarily and the volume curves obtained during their stimulation being superimposable. The results of previous workers are however quite explainable by Henderson's uniformity of behavior conception. The ratio of systolic duration to cycle length calculated from his curve (Fig. 3) shows that in the shorter cycles, which stimulation of the accelerators would give, more marked changes in systole occur than in the longer cycles such as are obtained by stimulation of the vagus. From the literature, therefore, it is difficult to be sure whether the nerves act only by altering the heart rate and that the other changes follow Henderson's law, or whether they have in addition a direct influence on the inherent irritability and contractility of the ventricular muscle.

#### V. RESULTS—THE VARIATIONS OF THE DURATION OF SYSTOLE AND DIASTOLE UNDER DIFFERENT CARDIOVASCULAR CONDITIONS

1. *Natural Variations of Systole and Diastole from Beat to Beat.*—It has been previously recognized that in man systole and diastole undergo variations from beat to beat.<sup>48</sup> In fact Lombard and Cope noted that rhythmic alterations of these phases occurred synchronous with the respiratory and vasomotor changes. No attempt was made in the present report to enter into the detailed relation of the heart's phases to these phenomena. It was found, however, that in anesthetized dogs, under natural conditions, variations occur during consecutive cycles. The greatest and smallest values in 10-15 such cycles were tabulated in a few experiments (Table I). It is evident from this table that, as in man, diastole is more variable than systole. Dogs with the more rapid heart rate (animals 1, 2, 3) show less variation in the duration of diastole than those with longer cycles (animals 4, 5, 6) although systolic variations are about the same in both. It may be supposed that in these "short cycle" dogs the accelerator-vagus balance, in the sense of Reid Hunt,<sup>42</sup> is thrown over to the accelerator side because of a diminished vagus tone.

TABLE I  
THE VARIATIONS OF SYSTOLE AND DIASTOLE FROM BEAT TO BEAT UNDER STANDARD CONDITIONS\*

ANIMAL	SYSTOLE	DIASTOLE	VARIATIONS IN SYSTOLE	VARIATIONS IN DIASTOLE
1	.137-.145	.175-.192	.008	.017
2	.152-.165	.177-.192	.013	.015
3	.200-.210	.205-.217	.010	.012
4	.222-.227	.325-.350	.005	.025
5	.235-.250	.335-.385	.015	.060
6	.257-.262	.357-.410	.005	.053

\*In this, as in the following tables, representative cycles only are given for clarity.

After section of the vagi some variations of both systole and diastole still occur, as is illustrated in Table II, showing that the vagi are not the only influences rhythmically affecting the phases of the cardiac cycle. This suggests that normally, in addition to the vagus tone, some other tonic cardiac influences, such as the accelerator nerves, or some of the physical conditions associated with the contraction of the heart, are able to control the temporal variations of systole and diastole.

This conclusion is further indicated on studying the relation of systolic to diastolic variations. Thus, Lombard and Cope<sup>45</sup> recently found that in man the variations of these phases, as they measured them, were neither synchronous nor of like degree.

TABLE II

THE VARIATIONS OF SYSTOLE AND DIASTOLE FROM BEAT TO BEAT AFTER THE VAGI WERE CUT.

ANIMAL	SYSTOLE	DIASTOLE	VARIATIONS IN SYSTOLE	VARIATIONS IN DIASTOLE
1	.117-.135	.160-.177	.018	.017
2	.127-.137	.112-.167	.010	.025
3	.180-.200	.180-.197	.020	.017
4	.202-.215	.285-.297	.013	.012
5	.215-.220	.340-.357	.005	.017
6	.247-.257	.292-.327	.010	.035

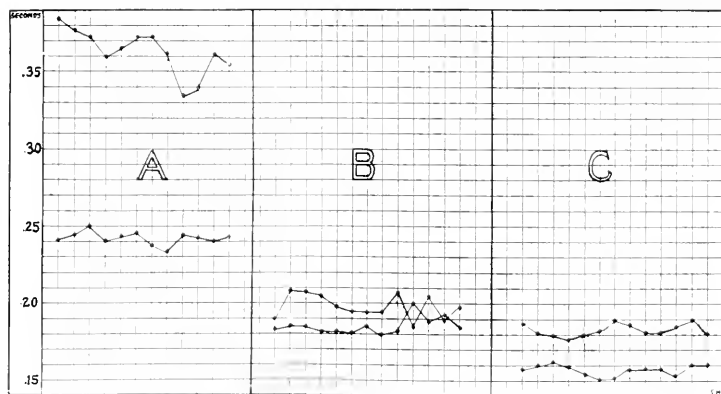


Fig. 4.—The relation of systole to the preceding diastole under natural conditions, each systole plotted as a dot vertically below its preceding diastole. Upper plot diastole, lower plot systole. A, B, C— from three different animals.

In studying the relation of these two phases, it was found more advantageous in this investigation to compare the duration of systole with the *preceding* diastole rather than with the one that follows. In this way one can determine whether systole varies independently of diastole or whether it follows the changes induced in the phase preceding it. No constant relation was found on carrying out this comparison in a number of experiments as the results of a few experiments illustrated in Fig. 4 show. These observations demonstrate that the variations of systole are not entirely determined by variations of diastole occa-

sioned by changing vagus tone. Although the variations are slight, yet often the alteration in systole is in the same direction as that of the following rather than the preceding diastole being in the opposite direction to the latter. It follows from this that in some cases at least the influence that causes the variation acts directly on systole (Fig. 4).

2. *Relation of the Duration of Systole to Heart Rate under Natural Conditions.*—A number of attempts have been made to show that a definite, or at any rate an average systole, normally occurs for any given heart rate. Lombard and Cope's formula, alluded to above, is an attempt to effect such a correlation in man. It is interesting to apply this formula to dogs and check its reliability.

This was attempted in Fig. 5 where the actual  $\frac{\text{systole}}{\text{cycle}}$  ratio (the ratio of systolic duration to cycle length) of the various animals under standard conditions is compared with the ratio calculated from Lombard and Cope's formula.

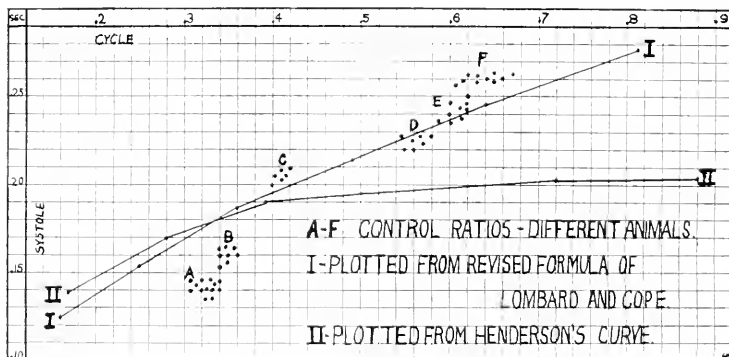


Fig. 5.—The relation of the normal systole-cycle ratio of several dogs, as compared with the curve plotted from Lombard and Cope's revised formula, ( $S = .31\sqrt{C}$ ) and with the curve plotted from Henderson's published curve (adding 0.05 sec. for the isometric period).

Inasmuch as such a plot involves the use of cycle length, whereas Lombard and Cope used heart rate, their formula was revised here by substituting  $C$  (cycle length) for  $R$ . It is obvious that the heart rate is the reciprocal of the cycle length. This may be expressed by the equation  $R$  (heart rate per minute) =

$$\frac{60}{C} \text{ (cycle length in seconds)} \quad \text{On substituting } \frac{60}{C} \text{ for } R, \text{ their formula } S = \frac{60}{K\sqrt{R}} \text{ is changed to } S = \frac{60}{K\sqrt{\frac{60}{C}}}$$

are first squared  $S^2 = \frac{60^2}{K^2 \frac{60}{C}}$  or  $\frac{60 C}{K^2}$  resulting. Extracting the square

root of this last equation changes it to  $S = \frac{\sqrt{60C}}{K}$  or approximately  $S = \frac{7.8\sqrt{C}}{K}$ . Since  $K$  for the recumbent position is 25 the equation can be further simplified



to  $S = .31\sqrt{C}$ . It is evident from the plot (Fig. 5) that Lombard and Cope's formula holds fairly accurately in dogs whose heart-rate is less than 150 per minute. In the more rapid rates, however, the systole-cycle ratio actually found falls short of that calculated from their formula. This indicates that although such formulae may be very valuable in calculations for which they are intended, yet it is difficult to evolve a formula that will take cognizance of all conditions and be applicable without limit.

Another attempt to relate systole and cycle length may be founded on the standard volume curve of Henderson (Fig. 3). This was attempted as illustrated in Fig. 5, where the actual systole-cycle ratios of several dogs under standard conditions were compared with the ratio calculated from Henderson's published curve. The plot shows that the actual results do not agree with the ratio calculated from his curve, making it obvious that the standard volume curve of one animal is not applicable to another. To establish a theoretical systole-cycle ratio with which to compare the actual results it is therefore necessary to construct a standard curve for each animal. A detailed and illustrated presentation of the manner of constructing such a curve has already been published with Wiggers.<sup>42</sup> A brief outline of the method may, however, be presented here. A long vagal beat, occurring after slowing has been established for some time, is selected. From it the duration of its ejection period is determined and laid off on the abscissa of a large-sized coordinate paper. An arc, similar in contour to that given in Henderson's curve, is drawn to fill in this time. At one second intervals on the abscissæ, segments of arcs of the same contour and parallel to the ejection curves are erected. The duration of the ejection periods at different cycle lengths and for different filling curves are then determined and from these a curve of systole-cycle ratios is plotted as the standard for comparisons in that animal.

*3. Relation of Consecutive Systolic and Diastolic Durations when the Cardiovascular Conditions are in the Process of Changing.* (a) *Relation During Changing Heart Rate:* It is interesting to further determine how the duration of systole and diastole alter with changing heart rate. The experiments performed in this research show that when the heart rate increases from a slow to a more natural rhythm, such as occurs in the recovery from vagus stimulation, (Fig. 6), the period of diastole shortens below *normal* in a short time. Systole however does not decrease so rapidly and reaches normal somewhat later. Again, when the heart rate changes from a rapid to a more natural rate, such as occurs after the cessation of accelerator stimulation (Fig. 6) diastole immediately increases above the *normal* duration while systole may not return to its former duration until twenty-five seconds later. These observations suggest that during the alteration of heart rate the duration of diastole is affected first. However, when the accelerators act as during the beginning of epinephrin action (Fig. 6) and during the early part of electrical stimulation of these nerves, systole is occasionally, but not always, shortened first.

It is obvious from these observations that, because of a lack of a constant relation between systole and diastole, the systole-cycle ratio will vary, depending on whether cycles are taken during the early or later stages of a change. It is

during these periods of changing heart rate that cycles which deviate noticeably from Henderson's law of Uniformity of Behavior are found.

In a later section of this report the temporal changes produced by stimulation of the vagi and accelerator nerves will be analyzed. There are, however, noticeable differences when such stimulation ceases. Thus, after the vagus has ceased to act, the heart rate—which is inversely proportional to cycle length—quickens for a time beyond normal, as pointed out above. This is explainable either on Gaskell's hypothesis<sup>15</sup> that the vagi are anabolic and have caused an excess of energy to be stored up or that their normal tone is lost, due to the exhaustion from the stimulation. The lagging of systole can be further explained by the increase in diastolic volume of the ventricle due to the decreased minute output occurring during the stimulation. The increase of diastolic volume outlasts the stimulation and this will maintain the increased duration of systole.<sup>51</sup> As the rate becomes more rapid and the minute output again in-

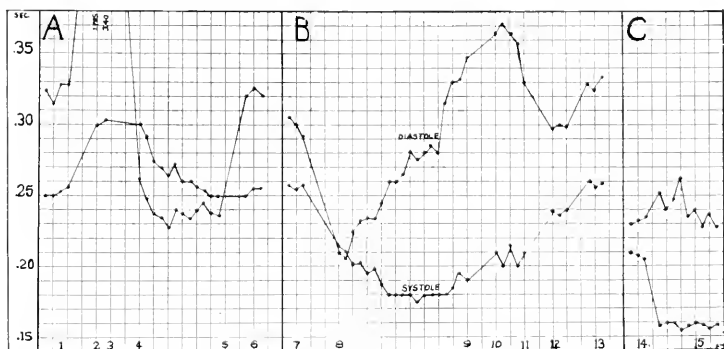


Fig. 6.—The variations of systole and diastole: A—during and after vagus stimulation; B—during and after accelerator stimulation; C—during the action of epinephrin. 1—control; 2,3—during vagus action; 3—vagus stimulation ceased; 3,4—six beats omitted; 4,5—after cessation of vagus stimulation; 6—control; 7—control; 8—the 58th beat after onset of accelerator stimulation; 9—stimulation ceased; 9,10—ten beats omitted; 11,12—35 beats omitted; 10,11, 12—after cessation of accelerator stimulation; 13—control; 14—control; 15—early during the action of epinephrin.

creases, the diastolic volume returns to normal and, as a result, systole again becomes of normal duration.

When considering the results of recovery from the accelerator stimulation, pointed out above, the slowing of the rate beyond normal can be explained by assuming that these nerves are catabolic,<sup>15</sup> or that the stimulation has exhausted them and so removed their normal tonicity. The marked lagging of systole indicates that, in addition to the influence of changing diastolic volume, there must be some specific effect on the heart muscle by these nerves. The more prompt effect on systole sometimes produced by epinephrin and by stimulation of the accelerators, pointed out above, is further evidence for this conclusion.

(b) *Relation During Changing Venous Pressure*: Temporary variations of the duration of systole which occur with changing heart rate may also appear during the modification of other cardiovascular influences, such, for example, as the venous pressure. The changes occurring during increasing venous pres-

sure can be readily produced by a rapid intravenous injection of saline. This was done in a number of experiments after the vagi were sectioned and the variations during and after the injection determined. The results of one such experiment, typical of all, is illustrated in Fig. 7. From this figure it will be seen that the changes produced vary, depending on how soon after the injection the cycles are taken. Thus, the sudden increase in venous return first causes a prolongation of systole and a decrease in the duration of diastole, the heart rate remaining unaltered.

The fact that the mean arterial blood pressure does not increase at this stage, as is the case when the minute output is increased, leads to the conclusion that the heart does not respond at once with an increased output. Blood therefore accumulates in the ventricle leading to its dilatation. The increased diastolic volume thus produced may account for the prolongation of systole.<sup>51</sup>

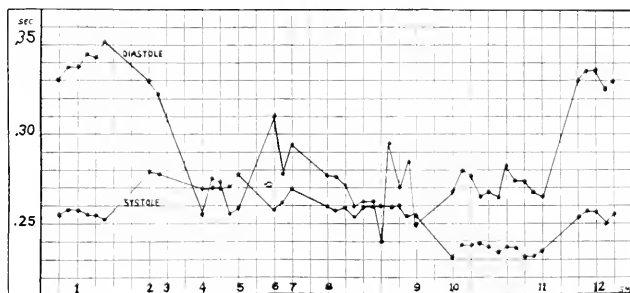


Fig. 7.—Relation of systole to diastole during various stages of saline infusion. 1—control, mean blood pressure (B.P.) 100, heart rate (H.R.) 100; 2—18th beat after injection of saline; 2.3—B. P. 110, H. R. 100; 3.4—24 beats omitted; 4.5—B. P. 102, H. R. 115; 5.6—29 beats omitted; 6.7—B. P. 110, H. R. 110; 7.8—10 beats omitted; 8.9—B.P. 124, H.R. 115; 9.10—50 beats after injection of saline stopped; 10.11—B.P. 128, H.R. 119; 12—control several minutes later B.P. 100, H.R. 100.

The mean blood pressure soon rises however showing that the heart, even in the absence of the vagi, has begun to compensate by increasing the minute output. This is accomplished in part by an increased heart rate and, perhaps, also by an increased systolic discharge. Systole during this stage continues to be longer than the theoretical values for these rates being approximately equal to the longer control cycles. The prolongation of systole chiefly affects the ejection phase, as was pointed out in reviewing the literature, and this would be an additional factor in increasing the systolic discharge.

The heart rate remains rapid and the blood pressure is maintained high for some time after the cessation of the injection, implying that the plethora induced by the rapid intravenous infusion has persisted. The duration of systole, however, does not remain prolonged but becomes equal to the theoretical value even while the heart rate and blood pressure are still affected.

As these observations show, the cycles obtained during rapid intravenous injection of saline deviate from Henderson's uniformity of behavior law.

4. *The Ultimate Effect of Altered Cardiovascular Changes on the Duration of Systole and Diastole*: In studying the effect of any influence on the temporal relation of the cardiac phases, cognizance must be taken of the temporary changes which occur while the heart is accommodating itself to the new conditions. An analysis of the temporary changes occurring in the recovery from nerve stimulation and during the intravenous infusion of saline was presented above. Similar changes may occur when any other factor associated with the cardiac cycle is modified. These temporary changes must be excluded when the ultimate effects produced by a certain influence are studied. This is not an easy task for, as under natural conditions, slight variations of systole and diastole occur also after the heart has accommodated itself to the new conditions.

In selecting the cycles to determine the end effects two criteria were used. In the first place cycles were selected which occurred a sufficient interval after the onset of the change, the number of beats excluded varying directly with the amount of departure from normal. However, in order to avoid the cycles occurring when the deviation was returning to normal too long an interval was not allowed to elapse. A second criterion depended on the amount of variation occurring in the consecutive cycles selected. The variations in these cycles were less than those occurring in successive cycles during the temporary changes. Although no arbitrary amount of variation was set yet the actual variation of the 10-15 cycles selected approximately equaled that occurring in successive cycles under natural conditions.

(a) *Ultimate Effect of Altered Mechanical Influences on the Temporal Length of Systole and Diastole*: As it is essential to exclude as carefully as possible any influence other than the one studied, the vagi which are the main channel for compensation were divided. For technical reasons, however, no attempt was made to exclude the accelerator nerves. The two main mechanical influences capable of modifying the cardiac action are (1) the venous pressure and (2) the arterial resistance.

(1) *Increased Venous Pressure*: An investigation of the effects of increased intracardiac volume, is of some clinical value because it may increase our understanding of the causes of cardiac dilatation. Cardiac dilatation occurs in many clinical conditions. Thus, chronic dilatation is found in many cases of chronic myocarditis, due to lues, chronic intoxications, or as the sequel of such acute infections as rheumatic fever, scarlatina, diphtheria, etc. Acute dilatations occur in many hearts during fevers, in cases with degenerated myocardiums and even in normal hearts as a result of severe unaccustomed exercise. The causes of the dilatation in these conditions are still imperfectly understood.<sup>11, 20</sup> These dilatations are frequently associated with an increased venous pressure.

An increase in the intracardiac volume was produced in these experiments by raising the venous pressure. The manner in which an increase in venous pressure produces the dilatation has already been discussed in considering the temporary effects of this condition. An increased venous pressure was produced by permitting saline to flow rapidly into the external jugular vein from a reservoir placed 120 cm. above the animal. Three experiments typical of all were selected (Table III) to show the response of a normal heart to a rapid increase in venous return.

The results show that systole is lengthened to a variable extent while diastole is either shortened (Exp. 3, Table III), lengthened (Exp. 2), or unchanged (Exp. 1), as a result of saline injection. When a larger amount of saline was injected irregularities of the heart beat occurred.

TABLE III

THE EFFECT ON SYSTOLE AND DIASTOLE OF INTRAVENOUS INJECTION OF SALINE (VAGI SECTIONED)

	EXPERIMENT 1		EXPERIMENT 2		EXPERIMENT 3	
	SYSTOLE	DIASTOLE	SYSTOLE	DIASTOLE	SYSTOLE	DIASTOLE
Standard	.202	.215	.21	.275	.255	.34
					.28	.33
During Injection	.227	.232	.225	.305	.27	.255
of Saline					.26	.26
After Injection	.20	.23	.21	.31	.237	.265

The variability in the response of the heart to superadded work and the rapidity of the injection modifies the amount of systolic lengthening. In the absence of the vagus control all the modifications above noted are produced either locally or through the accelerators. The local action modifying the heart rate, and perhaps slightly the duration of systole, is either determined by alterations in the nutrition of the sinus node and heart muscle or by the altered mechanical conditions produced by a change of intraventricular pressure. The main cause of the temporal lengthening of systole, however, is probably the increase in the diastolic volume and initial pressure of the ventricle produced by the increased venous pressure.

(2) *Increased Arterial Resistance*: An increase in arterial resistance occurs clinically, notably in such conditions as aortic stenosis, arteriosclerosis or compression of the aorta by a neoplasm or aneurysm. The prolongation of systole in the first condition has been clinically noticed and also shown to occur when aortic stenosis is produced experimentally (de Heer<sup>38</sup>). An increase in arterial blood pressure may be readily induced by compressing the abdominal aorta. This was accomplished by applying digital pressure to the aorta, the fingers being introduced through a small incision in the anterior abdominal wall. When this was done the results in most instances were not marked, inasmuch as the increased resistance thus produced was not great enough. In one case, however, when the common carotid was blocked at the same time a striking change was noted. As can be seen from Table IV the increase in arterial resistance caused a lengthening of systole although the heart rate, in the absence of vagus influence, remained the same.

TABLE IV

THE EFFECT OF COMPRESSION OF THE ABDOMINAL AOETA AND COMMON CAROTID ON SYSTOLE, DIASTOLE AND BLOOD PRESSURE (VAGI CUT)

SYSTOLE	CYCLE	DIASTOLE	BLOOD PRESSURE	
.18	.43	.25	84 mm.	Control
.205	.42	.215	120 mm.	During compression
.165	.42	.235	72 mm.	After release

It follows from these observations that in normal animals with sectioned vagi as in the heart-lung preparations,<sup>51</sup> the duration of systole can be altered by certain mechanical factors independent of changes in heart rate.

(b) *The Ultimate Effect of Stimulation of the Vagus and Accelerator Nerves on the Duration of the Cardiac Phases:* The results of these experiments have already been reported in detail in a previous paper in association with Wiggers<sup>62</sup> so that only a summary will be presented here. The end results of stimulation of these nerves were compared with the standard curve of systole-eye ratio established for this animal and it was found that (1) vagus stimulation increases systole and diastole, the latter more than the former, and the systole-eye ratio conforms to the theoretical curve; (2) vagus section causes a shortening of systole and diastole, the systole-eye ratio conforming to the theoretical line, although falling slightly below it; (3) stimulation of accelerators, both before and after the vagi are cut, causes a marked shortening of systole and diastole and the systole-eye ratio is *very noticeably below* the theoretical values for those eye lengths; (4) during stimulation of the vagus center by pituitary extract, accelerator stimulation causes a marked shortening of systole without any appreciable effect on diastole, the systole-eye ratio again falling noticeably below the theoretical values.

The conclusions drawn from these observations are that the vagi have no direct effect on the ventricular muscle, the changes in systolic durations being accountable on the variation of diastolic volume at different heart rates. The accelerators, on the other hand, through some selective influence on the inherent irritability and contractility of the ventricle, exert some specific action on the duration of systole.

(c) *The Ultimate Effect of Epinephrin on the Duration of Systole:* So far an analysis of the effect of different single influences has been attempted. Any change that occurs in the living body, however, is the result of alterations of several factors. It is therefore desirable to determine the effect of experimentally modifying more than one influence at the same time.

Epinephrin offers a ready method of doing this. A discussion of its actions on the circulation is given by Sollmann.<sup>16</sup> The injection of epinephrin causes a rise of the mean arterial blood pressure by stimulating the vasomotor endings. When the vagi are intact, the rise of blood pressure stimulates the vagus center overcoming, in this way, any effect the epinephrin may have on the accelerator nerves. On sectioning the vagi, however, only the accelerator stimulation remains. The injection of epinephrin at this time produces an increase in the heart rate and a more marked rise of the blood pressure. The observations already presented in this report demonstrated that accelerator stimulation produces a marked shortening of systole and that following a sharp rise of blood pressure this period is noticeably prolonged. It is therefore interesting to see what is the final result of producing these changes simultaneously.

The result of one such experiment is illustrated in Fig. 6 of a previous report with Wiggers.<sup>62</sup> This figure shows that the actual systole-eye ratio falls markedly below the theoretical curve. It is obvious from these results that under these conditions, the effect produced by epinephrin action parallels that obtained with electrical stimulation of the accelerator nerves; that is, epinephrin, through its accelerator stimulation, exerts a selective action on systole sufficient to overbalance the effect of increased resistance produced by the high blood pressure.

## VI. SUMMARY AND CONCLUSIONS

The temporal variations of the cardiac phases in dogs with normal circulations were determined from optical records of the heart sounds.

It was found that under natural conditions diastole is more variable than systole. The duration of diastole depends mainly, but not entirely, on vagus tonus. The variations of systole bear no constant relation to the preceding diastole, being even less affected than diastole by the vagus tonus.

No entirely successful method of relating the duration of systole to heart rate has as yet been found. Lombard and Cope's formula, an attempt in this direction, although fairly accurate in dogs at rates slower than 150 per minute, is quite inexact at more rapid rates. An attempt to relate systole to heart rate by means of a volume curve such as Henderson's on the assumption that the heart beats according to a uniform plan showed that a different curve of this kind must be constructed for each animal.

A corollary to Henderson's conception is that the duration of systole and diastole bear a fixed relation to the heart rate. Although many of the results of other investigators may be accounted for in this way, many others are apparently contradictory.

In studying the ultimate effect of a single influence it is essential to take account not only of the many other variable influences but also of any temporary changes that may be produced.

It was found that while the heart is changing from one rate to another due to varying nervous control, the duration of diastole alters first, except occasionally, during the beginning of epinephrin or accelerator stimulation. The cardiac periods do not follow the Uniformity of Behavior law during such changes in rate. This deviation is especially marked in the recovery from accelerator stimulation during which systole is abbreviated for a long time due to a persisting selective action of these nerves.

During the rapid intravenous injection of saline, which increases the venous pressure, systole is prolonged independent of the cycle length. This is another example of a deviation from Henderson's Uniformity of Behavior conception.

The ultimate effect of mechanical influences, such as marked changes in venous pressure and arterial resistance, is the production of variations of systole independent of heart rate.

It appears from the results presented that the vagi modify the duration of systole mainly in so far as the change in heart rate produced affects the diastolic volume of the ventricle. The accelerator nerves, however, through a selective effect on the inherent irritability and contractility of the ventricle, exert a specific influence on the duration of systole.

Epinephrin, through its accelerator stimulation, produces so marked a selective action on the systolic duration that the contrary effect of the high arterial resistance is overbalanced.

Consequently the conclusions reached are: (1) the duration of systole is normally modified to a large extent by the heart rate according to a uniform cardiac action conception, such as Henderson presented; (2) other influences, however, for instance alterations of arterial resistance and venous pressure, the

specific action of the accelerator nerves and those occurring during changing heart rate and even naturally from beat to beat, are capable of producing deviations from this uniform action in an animal with a normal circulation.

#### VII. POSSIBLE CLINICAL APPLICATION OF RESULTS

The criterion used for diseases of the heart has changed in recent years. No longer are cardiac diseases entirely classified according to the murmur heard, but, as MacKenzie<sup>11</sup> has emphasized, on the functional integrity of the myocardium. It is not difficult to diagnose a case of advanced cardiac failure. However, if very much good is to be done a much earlier diagnosis is desirable. Such an early diagnosis has been worked out on the basis of dyspnea, which we are told is one of the earliest clinical signs of cardiac failure. It would be advisable for general purposes such, for example, as to determine operative or insurance risks to get even an earlier sign than this. The possibility of establishing such a sign from the durations of the cardiac phases demands further investigation.

A detailed study of the temporal lengths of these phases in recognized and suspected cardiac diseases would be interesting and desirable. It may be found, for instance, that the duration of the cardiac phases in the diseased heart as compared to the normal heart, or the comparative changes induced in these periods by measured strains would give a method of determining the functional integrity of the myocardium. Only a few of some other possible applications of such investigations are indicated below.

1. Temporary or continued tachycardia is a clinical condition so frequently observed in association with other nervous symptoms that it has come to be looked upon as due to an abnormal balance of the cardiac control exerted by the vagi and accelerator nerves. The rate sometimes becomes so rapid that it is difficult to refer it entirely to lack of vagus tone but necessitates the assumption of an accelerator stimulation. The only hopeful way of determining whether the accelerators are really involved is to accurately measure the actual duration of systole in these conditions and compare it with the theoretical value determined from the standard volume curve of the individual.

2. Physiologically the pronounced shortening of systole may be looked upon as one means of preventing an excessively high arterial pressure with increasing heart rate. Thus, in an accelerated heart there is a tendency to prevent an increased minute output not only by a decreased diastolic filling but also by an abbreviation of the ejection period. It is conceivable that if the acceleration is produced in another manner (e.g. toxic substances affecting the sinus node) such a shortening does not occur. In this case a greater strain would be thrown on the vasomotor mechanism to prevent an abnormal elevation of pressure leading in this way to a resultant persistent dilatation. Furthermore, the abbreviation of systole induced by accelerator stimulation tends to spare the ventricular muscle from the contractile stress and in this way compensates for the more frequent occurrence of systole and the shortened diastoles. It is conceivable that this sparing influence of a shortened systole is lost when the increase in heart rate is not due to accelerator stimulation. Whether this may account for or contribute to certain of the symptoms, such as result from a weakened heart associated with some types of tachycardia, remains to be seen. Of significance in



this connection are the observations of Wiggers and Clough<sup>61</sup> who consistently found that the period of systole was of longer duration in functional cardiac disorders (irritable hearts, D. A. H.) than in normal individuals. If the premises presented in this and in the report published with Wiggers<sup>62</sup> are correct, it would indicate that the rapid hearts in these conditions are not due to a hyperexcitable sympathetic system.

3. The lengthening of systole found in cases of aortic stenosis has been variously explained, as due to a compensatory mechanism of the ventricle to permit sufficient blood to flow through the stenosed opening. If the assumption that systole can be modified by the diastolic volume is correct, then the mechanism of this compensation can be readily explained, as the stenosis itself is capable of producing such an increase in the diastolic volume. The contrary statement sometimes made that in aortic insufficiency the period of systole is decreased would, on the face of it, be incompatible with this conception, as in this condition also an increase in diastolic volume occurs. However, in all such cases the shortening of systole may be explained on the basis of an increased heart rate. In any event, the methods that have been used to determine the duration of systole were too inaccurate to be very valuable. The writer is unaware of any accurate study of this problem which merits further investigation.

4. The adaptation of the heart to varying conditions is well illustrated in the response to added work. When more blood returns to the ventricle, it responds by expelling more blood, not only by a greater number of ejection periods, but also by a greater relative duration of each systole. In certain diseased conditions, however, the heart muscle is so weakened that it cannot respond to this added strain and "heart failure" ensues. Whether in these conditions the duration of systole is lengthened remains to be seen.

Many other possible clinical problems suggest themselves but the few mentioned are sufficient to show that further investigations along these lines would be of practical clinical value.

This investigation was performed in the physiology laboratory of the Western Reserve University Medical School, under the supervision of Professor C. J. Wiggers. I wish to express my thanks to Dr. Wiggers for his advice and suggestions.

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## CLASSIFICATION OF STREPTOCOCCUS\*

### III. STREPTOCOCCI FROM NORMAL AND PATHOGENIC THROATS, ALSO FROM WOUNDS, CLASSIFIED BY SUGAR FERMENTATIONS, LIMITING HYDROGEN-ION CONCENTRATION, AND REACTIONS ON MILK MEDIUMS

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IN the former papers we have considered the classification of streptococci, based principally upon their ability to ferment certain sugars. We found the streptococci in the average person's throat to be remarkably constant in health and during attacks of acute rhinitis and pharyngitis and also during a recurrent "influenza" attack.

In the present paper, parallel with the sugar fermentations, the limiting hydrogen-ion concentration and certain reactions on milk mediums have been observed.

#### HISTORICAL

Broadhurst<sup>9</sup>, Stowell, Hilliard and Schlesinger<sup>25</sup> and Smith and Brown<sup>24</sup> record a difference in the acidity produced on dextrosebenillon between strains of streptococci from human and bovine sources, the acidity being determined by titration, using standard sodium hydroxide solutions and phenolphthalein as the indicator. Ayers<sup>4</sup> found the limiting hydrogen-ion concentration, determined colorimetrically, of streptococci from human infections to range between  $P_H$  5.5 and 6.0, this held true for 26 strains of the 34 tested; those strains isolated from human mouth, and from cow's milk, mouth, udder and feces had a  $P_H$  4.6 to 4.8 limiting hydrogen-ion concentration (151 of 167 cultures). Smillie,<sup>23</sup> using Henderson and Palmers<sup>14</sup> colorimetric method for the hydrogen-ion determination, found 4 strains of hemolytic streptococci isolated from the throats of scarlet fever cases to have a limiting hydrogen-ion concentration of  $P_H$  5.1 to 5.7 and one strain a value of  $P_H$  4.5. The former 4 strains proved to be pathogenic for mice, the latter strain was nonpathogenic. Avery and Cullen<sup>2</sup> obtained, colorimetrically, a  $P_H$  5.0 to 5.3 for 124 human strains and a  $P_H$  4.3 to 4.5 for 45 bovine strains. Brown<sup>8</sup> records a  $P_H$  4.5 to 4.6 for 12 bovine strains and a  $P_H$  5.1 to 5.4 for 18 presumably human strains. Jones, F. S.<sup>16</sup> isolated 56 strains of hemolytic streptococci from milk and found that these strains fell into two main groups, 43 produced a limiting hydrogen-ion concentration of  $P_H$  4.4 to 4.7 and 13 a lower value of  $P_H$  5.0 to 5.2. Five cultures of the last group were tested as to their pathogenicity for rabbits, the results were all negative. Jones, F. S.<sup>17</sup> in another article records the limiting hydrogen-ion concentration of 7 human strains varying between  $P_H$  5.4-5.6; 5 low acid-producing strains from milk  $P_H$  5.1-5.8; 5 equine streptococci (4 strains isolated from the nasal mucosa and pharynx of horses suffering from influenza, 1 was cultivated from an abscess) produced

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$P_H$  4.7-4.9 and 7 bovine strains (mastitis)  $P_H$  4.6. All these four groups were grown on 1 per cent dextrose-bouillon. This author has carefully compared the titration with the colorimetric method for determining acidity of streptococci cultures upon various mediums. For the former he used 0.05 N sodium hydroxide solution. He shows the two methods to run remarkably parallel, in his hands the titration method proved a satisfactory method of distinguishing the degree of acidity of the cultures. Ayers, Johnson and Davis<sup>7</sup> were unable, with their dextrose-yeast-peptone medium, to distinguish between human and bovine strains by the titration method. Jones, F. S.<sup>17</sup> and Jones, H.<sup>18</sup> have both shown that the addition of serum to plain bouillon causes the streptococci to produce more acid than when grown on plain dextrose-bouillon alone. Broadhurst<sup>10</sup> called attention to the fact that streptococci grown on meat bouillon produced much more acid than when grown on meat extract bouillon. Avery and Cullen<sup>2</sup> found that pneumococci grown on several different sugars, in 1 per cent concentration, for 18 hours, produced the same final hydrogen-ion concentration.

Brown<sup>8</sup> observed that salicin was fermented slower than other sugars by streptococci in his series. Jones, H.<sup>18</sup> has shown that there is a difference between the limiting hydrogen-ion concentration for streptococci grown for four days on 1 per cent dextrose and 1 per cent lactose bouillon. This same author after studying the effect of various medium constituents upon the limiting hydrogen-ion value of streptococci concludes in part: "The amount of glucose which a given organism can consume is influenced by the buffer content of the medium, i.e., by such constituents as phosphates, protein, etc., which aid in holding the concentration of the hydrogen-ion from the toxic limit, thus permitting a larger amount of the sugar to be decomposed. An initial reaction with a  $P_H$  well over the alkaline side has the same effect."

In reviewing the literature, it seems that the action of streptococci on milk cultures has not yielded any information leading toward a differentiation between strains of this group. Recently Sherman and Albus<sup>22</sup> have compared 50 strains of streptococci, isolated from milk, with 50 strains isolated from the udder by drawing the milk directly into sterile flasks. These workers lay emphasis upon the acid production and coagulation of milk and upon the reduction of certain dyes, especially methylene blue. Their *Streptococcus lactis* group (from milk) caused curdling of the milk and reduction of the methylene blue within 24 hours. Their *Streptococcus pyogenes* group (from the udder) were not so regular in their curdling action and never reduced methylene blue milk cultures. Brown<sup>8</sup> did not find either coagulation or reduction of methylene blue milk of any great value in differentiation of strains. He records 50 per cent of the bovine strains coagulated milk within 24 hours, and that none of the human strains caused coagulation within this time, as to the methylene blue test, the reduction was not constant for either group.

#### TECHNIC

With the exception of a few changes in details, the technic was the same as in our former papers.<sup>1</sup> The blood agar was prepared in the same way. De-Kruif and Ireland<sup>12</sup> have recently recommended a sheep-serum-blood cell mixture, consisting of 20 per cent serum, 5 per cent cells and agar 75 per cent. This

method is based on experimental evidence of the maximum streptolysin production. Their technic was carried out on several groups of plates. If the agar is cooled properly, the blood added slowly while the container is well shaken during the process, under these conditions we have observed the minimum hemagglutination, making a clear uniform plate. With the DeKruif-Ireland technic there is a difference in the size of the hemolytic zones, but it is a quantitative difference only, the advantage of this technic—in our hands—has not been sufficient to warrant the additional work necessary to carry through their complicated technic.

The vitamine-bouillon was prepared as in our former work. There was an initial hydrogen-ion concentration of  $P_H$  7.8 for all bouillon cultures, determined by Barnett and Chapman's method<sup>6</sup> using phenol red as indicator. The growth was much better than on the bouillon adjusted to  $P_H$  7.0. In fact growth was so much heavier after 24 hours incubation as compared with the former bouillon of  $P_H$  7.0, that we worked some time eliminating all possibilities of contamination causing the increased amount of growth. In the protocols to be reported in this paper there is not a single "no growth" notation.

Lactose, mannite and salicin were used in 1 per cent concentrations in the vitamine-bouillon, each being tubed in about 5 c.c. quantities. The dextrose was in the same concentration, but about 8 c.c. was put into each tube. Care was taken to see that the reaction was  $P_H$  7.8 after the final sterilization. In view of the work done by Mudge<sup>20</sup> we have autoclaved all sugar mediums at 15 pounds for 15 minutes instead of sterilizing in the Arnold-sterilizer for three successive days.

The brom-cresol-purple milk was prepared according to Clark and Lubs,<sup>11</sup> this was used for detecting acidity and coagulation. The methylene blue milk was prepared with the same concentration of the dye as Sherman and Albus<sup>22</sup> recommended. We found it unnecessary to sterilize the two separately. The required amount of methylene blue can be added to the milk, then tubed and sterilized. After cooling, the tubes are shaken and the methylene blue is oxidized, giving the original colored milk solution.

In detail the technic was as follows: Swabs were directly touched to cooled and partially dried blood agar plates, then smeared well with a sterile bent glass spreader. If time did not permit or blood agar plates were not ready, the swab was immediately soaked for one minute in Holman's cooked meat medium,<sup>15</sup> this was always kept in the incubator ready for use. If the plates were to be inoculated within six hours, these warm Holman meat tubes were incubated, but if the material was not to be plated until the next day, the tubes were set aside and incubated for six hours just before plating. A loopful of this Holman meat culture was diluted with 5 c.c. of sterile Ringer's solution and after shaking well one loopful of this diluted suspension was plated, this was spread over the plate by means of the sterile glass spreader. If the swab was from a wound, two or more plates were usually smeared with the same spreader, after smearing the first plate, thereby getting a graduated dilution, insuring isolated colonies on the plates. These plates were inverted and incubated for twenty-four hours. The growth was described, and typical colonies were picked, in some cases 20 colonies from the original swab. The picked colonies were put on warm Hol-

man's meat medium and incubated for 6 to 24 hours, the time of incubation being of no importance so far as the morphology of the streptococci is concerned. After six or eight hours there is a good growth. These were stained, direct from the Holman meat medium, by Gram method. We have used the Weigert's gentian violet solutions, being unable to obtain the aniline sulphate for the permanent gentian violet solution as recommended by the Committee on Descriptive Charts.<sup>21</sup> The quick method for the Gram stain was used. All strains were classified morphologically, as to size of cocci, length of chains, appearance of the cocci in chains, i.e., if in diplococci form or single, elongated, etc. All diplococci in very short chains or appearing singly were eliminated, as so far these have been bile soluble and have added unnecessarily to the technical work. So far we have not found any of the bile insoluble strains described by Mair.<sup>19</sup> The streptococci proving to be in pure cultures were transferred to vitamine-bouillon, 1 per cent dextrose, lactose, mannite and salicin in vitamine-bouillon, and brom-eresol-purple and methylene blue milk, all these were inoculated from the Holman meat tube. The plain bouillon culture was used for the bile test. One drop of a 0.1 per cent alcoholic solution of the methyl red was added to each of the lactose, mannite and salicin tubes, any color from a salmon to a red was recorded as positive. Five c.c. was pipetted from the dextrose bouillon 48 hour culture into a clean test tube (150x20) one drop of an alcoholic solution of methyl red added. (We have found the amount, as recommended by Avery and Cullen,<sup>2</sup> 0.02 c.c. to give the sharpest end point, the pipette should be roughly standardized to drop 50 drops to the c.c.). To this is now added 10 c.c. of distilled water. A red color denotes a hydrogen-ion value of about 4.3 to 4.6, a salmon color  $P_H$  5.0 to 5.4. These were checked with the colorimetric method, the standards being prepared according to Clark and Lubs.<sup>12</sup> With a little experience the color obtained by the above technic can be read off to within 0.2  $P_H$  readily. There were a few cultures, as was mentioned by Avery and Cullen, that did not give any color at all with the methyl red indicator on the dextrose bouillon cultures, these are recorded as "irregulars" in the following tables. Several strains were found to rapidly decolorize the methyl red, first appearing as a deep red color, then after a few minutes a salmon and after 15 minutes completely decolorized to the original bouillon color. Jones<sup>17</sup> has observed the same action upon methyl red by some of his streptococci cultures. For this reason the methyl red was added to the sugars as an indicator for fermentation after they have been incubated. The colors were read immediately after addition of the indicator. The milk cultures were recorded after 12 hours, 24 hours and then every 24 hours for 8 days.

#### EXPERIMENTAL I. NORMAL AND PATHOLOGIC THROATS

One hundred thirty-nine strains of streptococci were isolated from the posterior nasopharynx of healthy persons, and from cases of acute tonsillitis and pharyngitis. There were 40 cases in all, twenty were apparently normal throats, and twenty from other than normal throats, the above diagnosis was given for the majority of these cases.

In the healthy throat series were 75 strains of streptococci isolated—42 hemolytic and 33 nonhemolytic strains.

## HEMOLYTIC STREPTOCOCCI

STRAINS WITH LIMITING H-ION CONCENTRATION OF

Holman's Classification	Number of		Ph.4.3-4.5	Ph.5.0-5.4	Irregular strains
	Strains	Per cent			
Streptococcus Anginosus	21	50.	20	1	0
Streptococcus Pyogenes	17	40.5	16	1	0
Streptococcus Subacidus	4	9.5	2	0	2

## NONHEMOLYTIC STREPTOCOCCI

Streptococcus Salivarius	20	60.6	17	2	1
Streptococcus Mitis	9	27.3	8	1	0
Streptococcus Ignavus	3	9.1	3	0	0
Streptococcus Equinus	1	3.	1	0	0

Throats showing evidence of acute inflammation. 64 strains were isolated, 27 hemolytic and 37 nonhemolytic.

## HEMOLYTIC STREPTOCOCCI

STRAINS WITH LIMITING H-ION CONCENTRATION OF

Holman's Classification	Number of		Ph.4.3-4.5	Ph.5.0-5.4	Irregular strains
	Strains	Per cent			
Streptococcus Anginosus	14	51.8	12	2	0
Streptococcus Pyogenes	9	33.4	9	0	0
Streptococcus Subacidus	3	11.1	2	1	0
Streptococcus Hemolyticus i	1	3.7	1	0	0

## NONHEMOLYTIC STREPTOCOCCI

Streptococcus Salivarius	22	59.5	21	1	0
Streptococcus Mitis	12	32.4	12	0	0
Streptococcus Ignavus	2	5.4	2	0	0
Streptococcus Nonhemolyticus i	1	2.7	1	0	0

These findings can be expressed in another way:

- 42 strains of hemolytic streptococci from normal throats:
- 38 strains or 90.48% would be classified as nonpathogenic, nonvirulent or bovine.
- 2 strains or 4.76% would be classified as pathogenic, virulent or human.
- 2 strains or 4.76% would not be classified (irregular).
- 33 strains of nonhemolytic streptococci from normal throats:
- 29 strains or 87.88% would be classified as nonpathogenic, nonvirulent or bovine.
- 3 strains or 9.09% would be classified as pathogenic, virulent or human.
- 1 strain or 4.03% could not be classified (irregular).
- 27 strains of hemolytic streptococci from pathogenic throats:
- 24 strains or 88.90% would be classified as nonpathogenic, nonvirulent or bovine.
- 3 strains or 11.10% would be classified as pathogenic, virulent or human.
- 37 strains of nonhemolytic streptococci from pathogenic throats:
- 36 strains or 97.30% would be classified as nonpathogenic, nonvirulent or bovine.
- 1 strain or 2.70% would be classified as pathogenic, virulent or human.

The following tables give the results of the 139 strains of streptococci on from-cresol-purple milk and methylene blue milk.



BROM-CRESOL-PURPLE MILK.	NORMAL THROATS, HEMOLYTIC.	PATHOLOGIC THROATS, HEMOLYTIC.
Acid within 24 hrs. coagulation after 24 hours.	9 strains—21.43%	9 strains—33.33%
Acid and coagulation within 24 hrs.	10 strains—23.81%	0
Acid and coagulation after 24 hrs.	10 strains—23.81%	9 strains—33.33%
Acid within 24 hrs. No coagulation.	3 strains—7.14%	4 strains—14.81%
Acid after 24 hrs. No coagulation.	9 strains—21.43%	4 strains—14.81%
No change.	1 strain—2.38%	1 strain—3.71%

BROM-CRESOL-PURPLE MILK.	NORMAL THROATS, NONHEMOLYTIC.	PATHOLOGIC THROATS, NONHEMOLYTIC.
Acid within 24 hrs. coagulation after 24 hrs.	13 strains—39.39%	13 strains—51.35%
Acid and coagulation within 24 hrs.	8 strains—24.24%	0
Acid and coagulation after 24 hrs.	8 strains—24.24%	11 strains—29.73%
Acid within 24 hrs. No coagulation.	0	2 strains—5.41%
Acid after 24 hrs. No coagulation.	3 strains—9.09%	3 strains—8.10%
No change.	1 strain—3.03%	2 strains—5.41%

METHYLENE BLUE MILK	NORMAL THROATS, HEMOLYTIC STREPTOCOCCI.	PATHOLOGIC THROATS, HEMOLYTIC STREPTOCOCCI.
Reduced within 24 hrs.		
Coagulation within 24 hrs.	3 strains—7.14%	0
Reduced within 24 hrs.		
Coagulation after 24 hrs.	6 strains—4.28%	2 strains—7.41%
Reduced after 24 hrs.		
Coagulation after 24 hrs.	14 strains—33.33%	3 strains—11.11%
Partial reduction.		
With or without coagulation.	0	5 strains—18.52%
Reduction.		
No coagulation.	1 strain—2.4%	0
No change.	18 strains—42.85%	17 strains—62.96%
Reduction before coagulation.		
Regardless of time factor.	13 strains—31. %	5 strains—18.52%
Reduction and coagulation at same reading, regardless of time factor.	11 strains—26. %	0

METHYLENE BLUE MILK	NORMAL THROATS, NONHEMOLYTIC STREPTOCOCCI.	PATHOLOGIC THROATS, NONHEMOLYTIC STREPTOCOCCI.
Reduced within 24 hrs.		
Coagulation within 24 hrs.	3 strains—9.09%	0
Reduced within 24 hrs.		
Coagulation after 24 hrs.	2 strains—6.06%	6 strains—16.22%
Reduced after 24 hrs.		
Coagulation after 24 hrs.	6 strains—18.18%	10 strains—27.02%
Partial reduction.		
No coagulation.	1 strain—3.03%	2 strains—5.40%
Coagulation.		

METHYLENE BLUE MILK	NORMAL THROATS, NONHEMOLYTIC STREPTOCOCCI,	PATHOLOGIC THROATS, NONHEMOLYTIC STREPTOCOCCI,
No reduction,	1 strain—3.03%	0
No change,	19 strains—57.57%	18 strains—48.65%
Reduction,		
No coagulation,	1 strain—3.03%	1 strain—2.70%
Reduction before coagulation regard- less of time factor,	10 strains—30.3%	10 strains—27. %
Reduction and coagulation at same reading, regardless of time factor,	4 strains—12. %	8 strains—22. %

## EXPERIMENTAL II. THROATS AND INFECTED WOUNDS OF THE SAME INDIVIDUAL

One case of severe streptococci infection of a scalp wound was studied. The infection spread over the whole scalp area and down on the left side of the face and neck. Cultures were made from the posterior nasopharynx and the infected wound the day the patient was admitted, and again six days later.

The results of the first culture were:

## POSTERIOR NASOPHARYNX

Streptococcus Subacidus	8 strains	Limiting H-ion	Concentration of Ph	5.2 to 5.5
" Subacidus	2 strains	" "	"	4.5
" Pyogenes	2 strains	" "	"	5.3
" Pyogenes	2 strains	" "	"	4.3
" Anginosus	2 strains	" "	"	5.3
" Ignavus	4 strains	" "	"	5.3

## WOUND

Streptococcus Pyogenes	4 strains	Limiting H-ion	Concentration of Ph	5.2 to 5.4
" Pyogenes	4 strains	" "	"	4.3 to 4.5

The second culture (six days later)

## POSTERIOR NASOPHARYNX

Streptococcus Anginosus	2 strains	Limiting H-ion	Concentration of Ph	5.4
" Subacidus	2 strains	" "	"	5.4
" Ignavus	2 strains	" "	"	5.3

## WOUND

Streptococcus Pyogenes	6 strains	Limiting H-ion	Concentration of Ph	5.3 to 5.4
" Mitis	2 strains	" "	"	5.4
" Ignavus	2 strains	" "	"	5.4

For the past year or so we have had in mind the question of the influence of throat streptococci upon those of the wound and vice versa. We have had the opportunity of studying only a few cases. These were typed only by Holman's classification, as the Avery and Cullen method using methyl red had not been published when this was done. There were 23 cases in this series, we are giving only ten cases here, which represent the average findings. These were isolated observations, made as time permitted and without choosing special cases.

## Case 200. Infected wound after removal of both breasts.

THROAT		WOUND	
<i>Streptococcus</i>	<i>Pyogenes</i> 3 strains	<i>Streptococcus</i>	<i>Ignavus</i> 2 strains
"	<i>Hemolyticus</i> i 1 strain	"	<i>Salivarius</i> 2 strains
"	<i>Ignavus</i> 2 strains		
"	<i>Salivarius</i> 2 strains		

## Case 200. Six days later.

THROAT		WOUND	
<i>Streptococcus</i>	<i>Mitis</i> 2 strains	<i>Streptococcus</i>	<i>Ignavus</i> 1 strain
"	<i>Salivarius</i> 1 strain	"	<i>Salivarius</i> 1 strain
		"	<i>Fecalis</i> 1 strain

## Case 202. Infected wound after removal of malignant growth from axilla.

THROAT		WOUND	
<i>Streptococcus</i>	<i>Hemolyticus</i> ii 2 strains	<i>Streptococcus</i>	<i>Hemolyticus</i> ii 1 strain
"	<i>Ignavus</i> 1 strain	"	<i>Salivarius</i> 1 strain
"	<i>Salivarius</i> 1 strain	"	<i>Fecalis</i> 1 strain

## Case 203. Draining pyothorax.

THROAT		WOUND	
<i>Streptococcus</i>	<i>Hemolyticus</i> i 2 strains	<i>Streptococcus</i>	<i>Equinus</i> 2 strains
"	<i>Mitis</i> 1 strain		

## Case 204. Infected gun-shot wound of arm.

THROAT		WOUND	
<i>Streptococcus</i>	<i>Anginosus</i> 1 strain	<i>Streptococcus</i>	<i>Infrequens</i> 1 strain
"	<i>Mitis</i> 4 strains	"	<i>Mitis</i> 2 strains

## Case 205. Infected gun-shot wound of foot.

THROAT		WOUND	
<i>Streptococcus</i>	<i>Equinus</i> 2 strains	<i>Streptococcus</i>	<i>Ignavus</i> 1 strain
"	<i>Mitis</i> 1 strain	"	<i>Salivarius</i> 1 strain
		"	<i>Fecalis</i> 1 strain

## Case 207. Otitis Media.

THROAT		WOUND	
<i>Streptococcus</i>	<i>Equinus</i> 2 strains	<i>Streptococcus</i>	<i>Equi</i> 2 strains
"	<i>Ignavus</i> 1 strain	"	<i>Subacidus</i> 1 strain
		"	<i>Nonhemolyticus</i> i 2 strains

## Case 209. Pus from hip fracture of 12 months' standing in elderly person.

THROAT		WOUND	
<i>Streptococcus</i>	<i>Salivarius</i> 4 strains	<i>Streptococcus</i>	<i>Nonhemolyticus</i> i 3 strains

## Case 216. Draining pyothorax.

THROAT		WOUND	
<i>Streptococcus</i>	<i>Anginosus</i> 1 strain	<i>Streptococcus</i>	<i>Equi</i> 1 strain
"	<i>Salivarius</i> 2 strains	"	<i>Salivarius</i> 1 strain

## Case 217. Draining pyothorax.

THROAT		WOUND	
<i>Streptococcus</i>	<i>Equi</i> 2 strains	<i>Streptococcus</i>	<i>Mitis</i> 2 strains
"	<i>Salivarius</i> 2 strains		

## DISCUSSION

The results of the first part of this paper seem to show that the streptococci, both hemolytic and nonhemolytic, in throats of cases of acute tonsillitis and pharyngitis do not differ, in their limiting hydrogen-ion concentration and fermentation of sugars, from those streptococci present in normal throats. Jones, F. S.<sup>17</sup> has isolated several strains of low-acid-producing streptococci from milk, the limiting hydrogen-ion concentration on plain dextrose-bonillon cultures ranging from  $P_H$  5.1 to 5.8. These strains if present in the throat and having this final hydrogen-ion concentration, would fall in the pathogenic group.

In the second part of this paper the only case of wound infection, that could be classified clinically as a virulent type, which was studied in respect to the limiting hydrogen-ion concentration of the isolated streptococci, presents many interesting features. There were hemolytic streptococci present in the throat of this patient during the first part of the infection that belonged to the pathogenic group ( $P_H$  5.0 to 5.3) and there were also some belonging to the nonpathogenic group ( $P_H$  4.3 to 4.5), both pathogenic and nonpathogenic giving the same sugar reactions, this was also true for the cultures made from the wound. Six days later all the strains isolated from both throat and wound were of the pathogenic variety. A swab only comes in contact with a relatively small amount of exudate and the swabs from such wounds are usually heavily seeded, as a result only a small portion of this is used for inoculation of the respective medium, consequently there is only a very small part of the exudate examined. The position in the wound of that portion of the exudate which is examined may exert considerable influence upon the streptococci isolated, i.e., whether the superficial or deep part of the exudate is collected on the swab, or even a swab taken directly from the wound surface after removal of the exudate. These points are now being studied in this laboratory.

The results we have obtained from the milk cultures have not yielded us anything that would help toward classifying the streptococci. The action of the brom-cresol-purple milk, as to the time of acid reaction and coagulation, and the methylene blue milk, as to the time of reduction and coagulation, seemed to have been independent of the sugar fermentations and limiting hydrogen-ion concentration on dextrose-bonillon. The factors underlying the reduction of the methylene blue in the milk cultures does not seem to be clearly understood. Bartel<sup>7</sup> seems to think the cause of the decolorization is explained by the exhaustion of the dissolved oxygen of the milk by the activity of the bacteria and the subsequent reduction of the lactose or other constituents of the milk. Several of our strains not fermenting lactose, readily reduced the methylene blue in the milk cultures. It probably is an indicator of a certain particular form of metabolism of the strain, involving the rate of oxygen requirement by the organism and possibly a reductase formation. This question is being further studied by one of us at the present time.

The use of the 1 per cent dextrose-bonillon for the sugar medium to be used for the limiting hydrogen-ion concentration has been found to be satisfactory. We have noticed a marked difference in the hydrogen-ion concentration in the different sugars used, after 48 hours incubation. The lactose, when fermented, seems to give about the same color as the dextrose, but the salicin and mannite

were found to vary within wide limits, usually giving a lower concentration of hydrogen-ions. The dextrose-bouillon medium was not fermented by some strains, or at least the hydrogen-ion concentration did not fall within the methyl red range. All these doubtful strains are being studied now by us. With more extensive work upon this group and the strains reaching a limiting hydrogen-ion concentration of  $P_H$  4.5 and  $P_H$  5.0, that is the upper limit of the nonpathogenic, nonvirulent or bovine group, and the lower limit of the pathogenic, virulent or human group, there may be some more light thrown on the subject of using the limiting hydrogen-ion concentration as a means of classifying streptococci.

One should also take into consideration that the work on the throat cases here recorded was done during the past summer months. This is being continued into the coming winter months, possibly there will be some differences observed, at least a parallel series will be of interest.

#### SUMMARY

The technic of the simple and rapid method of determining the hydrogen-ion concentration, recommended by Avery and Cullen, has proved satisfactory in our hands.

The hemolytic and nonhemolytic streptococci found in normal and pathogenic throats were of the same varieties, when classified by Holman's sugar fermentation tests. Avery and Cullen's final hydrogen-ion concentration and their action on brom-cresol-purple and methylene blue milks.

In many cases of infected wounds, particularly those of the head and upper extremity, the same strains of streptococci were found in the throat and the wound.

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## THE ETIOLOGY OF ACUTE INFLAMMATIONS OF THE NOSE, PHARYNX AND TONSILS\*

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(Continued from page 275.)

### CONCURRENT BACTERIOLOGIC STUDIES—SUMMER OF 1920

The bacteriologic methods of 1920 were essentially those of 1919 save that the baked blood plates for *B. influenzae* were omitted and that the vestibule of the nose was in each case washed out before the nasal culture with cotton wet with tap water.

*Results.*—Four subjects were used in this study. Cultures were made daily (with a few omissions) from June 21 to July 19 from the right and left nasal cavities, the right faucial tonsil, and the posterior pharyngeal wall of two subjects, S. M. and A. G. The third, S. B. G., was similarly cultured from June 21 to June 30, inclusive. The fourth, F. J. C., was cultured only once. S. M., A. G., and F. J. C. each developed a mild coryza. S. B. G. was unaffected.

Subject S. M. showed in the nose from the outset *Staphylococcus albus*. On June 24 and thereafter *S. aureus* also was found. Diphtheroids appeared in cultures from each nasal cavity made four hours after his third intranasal experiment. *S. albus* remained throughout all the experiments. *S. aureus* was present occasionally, but, except on the days following its first appearance, in smaller numbers than *S. albus*. The diphtheroids fluctuated in numbers and were sometimes absent. There was no apparent relation between their numbers and the chilling.

The tonsil contained at the outset *Streptococcus nonhemolyticus*, *pneumococcus*, and *S. albus*. *Streptococcus hemolyticus* appeared in the tonsil culture taken 24 hours after the first experiment on June 22; the applicator in this experiment was on the nasal septum and the subject's mouth was closed. Forty-eight hours after the experiment, a pure culture of *S. hemolyticus* appeared in the tonsil culture. The number diminished on the following day to 11 per cent of all colonies, and subsequently remained present in numbers from 1 to 12 per cent of all colonies counted, through July 12. Nose and throat remained clinically normal throughout this time. Further experiments were performed upon this subject July 12, 15, and 17, with the thermopile tips respectively on the nasopharyngeal and oropharyngeal wall and in the air of the postnasal space. The proportion of hemolytic streptococci in the tonsil cultures slowly rose during this time—July 12, 12 per cent; July 14, 16 per cent; July 16 and 17, numerous, not counted; July 19, 25 per cent; July 20, 36 per cent. July 17,

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this subject began to develop symptoms of coryza. By July 19 he had cough, nasal stuffiness and rhinorrhea and malaise. Symptoms present but abated the following day. The mother of this subject had had a severe cold from about July 12; his symptoms may or may not have been connected with the experiments.

The pharynx showed *Streptococcus nonhemolyticus* and *pneumococcus* and occasionally *S. albus* and *S. aureus*. June 27, *S. hemolyticus* appeared and subsequently it was obtained in four cultures, each time associated with tonsil cultures containing a like organism. *S. hemolyticus* is so usual an inhabitant of the tonsils (Davis, 1920) that its incidence here may or may not have been connected with the experiments.

Subject A. G. showed in the nasal cavity initially *S. aureus*; subsequently *S. albus* appeared on each side. The right side showed usually a preponderance of *S. albus*, the left of *S. aureus*. No other organisms appeared in the nasal cultures.

The right tonsil showed *Streptococcus nonhemolyticus* throughout, i. e., from June 21 to July 19. A. G. was subject of an experiment June 21, 23, and 24; application was made on the anterior end of the right nasal septum, the anterior end of the left lower turbinate and in the left middle meatus, respectively. The symptoms of a slight rhinitis—nasal stuffiness, slight headache and slight mucopurulent discharge—developed June 24. The secretion and stuffiness persisted until June 29. June 24 two colonies of *M. catarrhalis* appeared on the tonsil plate and June 26 one on the tonsil and three on the pharynx plate. June 28 *S. albus* began to be present in the right nose. Otherwise no change in bacteriology was noted, the nose showing *S. aureus* and the tonsil and pharynx nonhemolytic streptococci as before. A. G. was again subject July 7 and 17, with the applicator on the right middle turbinate in the first case and with no mucous membrane application at all in the second. July 17 twenty colonies (44 per cent) of *M. catarrhalis* appeared on the tonsil plate. There were no accompanying clinical symptoms. *Streptococcus hemolyticus* appeared after the experiment of July 7, four colonies on the tonsil and one on the pharynx plate July 7, one on the tonsil plate July 8 and one on the tonsil plate July 12. These were the only appearances of hemolytic streptococci in this subject either in the series of 1919 or 1920.

In subject S. B. G. there were present in the nose *S. aureus* and *S. albus*. On the right tonsil there were *S. nonhemolyticus*, *pneumococcus*, *S. aureus* and *S. albus*. The pharynx showed *S. nonhemolyticus* and *pneumococcus*. There was practically no change in the bacterial flora throughout the period studied neither were there any signs of a cold. The cultures from the pharynx were frequently sterile and always showed relatively few bacteria, as was the case in this subject in 1919.

F. J. C., whose pharyngeal culture showed abundant hemolytic streptococci, developed a mild cold the day following his first experiment (applicator on left nasal septum). Unfortunately a contagious origin cannot be ruled out, however, for his baby brother developed a cold about the same time.

*Discussion.*—*Streptococcus nonhemolyticus* was found in all four individuals studied, *pneumococcus* in two, *S. hemolyticus* in three, and *M. catarrhalis* in

one. In S. M., on one occasion there was an abundance of *S. hemolyticus* in the tonsil cultures before and during the symptoms of cold and sore throat. In F. J. C. a cold followed a single exposure of an individual with abundant hemolytic streptococci. The appearance of *M. catarrhalis* in A. G. after experimentation was paralleled by a like occurrence in 1919.

#### INTERPRETATION OF REACTIONS TO CHILLING

It is a fact beyond question that potentially pathogenic bacteria may lead a saprophytic existence upon the pharyngeal and tonsillar mucous membranes of healthy subjects (e. g., see Davis, 1920). It is equally indisputable that those bacteria may under appropriate circumstances become the active agents of infection, local or generalized. We believe that exposure to cold may be one such exciting factor of infection. We have shown also that chilling of the body surface causes a reflex vasoconstriction and ischemia in the mucous membranes of the nasal cavity, postnasal space, oropharynx, palate and tonsils. That the latter is the mechanism by which local resistance is lowered and infection excited we have not proved. However, there would seem to be justification for advancing tentatively, at least, the hypothesis that the ischemia may mediate the infection. Jonathan Wright in the course of a discussion of the etiology of acute upper respiratory infection (1914, p. 295), the most scholarly the writer has anywhere found, says:

"It may well be, as has been admitted, that certain bacteria are at once pathogenic when they reach the mucous membrane. Indeed, this seems very probable when they reach the mucous membranes of certain individuals. It may well be that such individuals always present, owing to systemic states, conditions of the mucosa which offer an ever-open avenue to infection; but granting all this, which indeed is in reality a part of our conception of the mechanism of the process, it seems extremely likely that local biochemical change, dependent upon molecular activities acting through the sympathetic nervous system, is the antecedent in the majority of cases of bacterial infection. This molecular disturbance of the normal activities of the sympathetic nerves may be set up by external or internal agencies, by the chilling of the body surfaces, or by derangements in the activities of the internal organs. Owing to the fact that wet feet and the chilling of distant regions of the surface of the body are, at least in clinical experience, quite as frequently followed by coryzas and sore throats as the direct impact of such external influences upon the head and neck, we have the right to infer that the shock at the surface must be transferred to internal ganglia and there translated into impulses which are carried to the surfaces of the mucosa of the upper air passages. There they give rise to the chain of biophysical and biochemical changes which may simply result in a mild coryza or a catarrhal pharyngitis, the resolution of which terminates the chain, or these conditions may be in themselves the starting point of bacterial invasion."

In the insufficient light of present knowledge, it would seem not improbable that the ischemia incident on cutaneous chilling, by decreasing cell respiration, or by retarding removal of the products of cell metabolism, or by increasing the permeability of the epithelial cell surfaces to the bacterial products, or by decreasing the local supply of specific antibodies, or by altering the media in the tonsillar crypts and folds of the pharyngeal mucosa in which the bacteria are living, or, especially when accompanied by direct chilling of the mucous membranes, by altering the state of aggregation of the colloids of the protoplasm (Schade, 1920) or by a combination of these factors, might affect the local



change postulated by Wright and thus so disturb the equilibrium between host and parasite as to excite infection. We here use the term "infection" as denoting a process separable from that of invasion or penetration of the bacteria into or through the mucosa. For study of tonsils in the early stages of infection has usually shown the crypts swarming with bacteria with none demonstrable beneath the mucosa surface (Wright and Smith, 1914, p. 291; Wright 1907; Goodale 1899). There is much collateral evidence to support the hypothesis of Wright that a factor other than any of those suggested above; namely, the surface-tension relations of bacteria and mucosa cell surfaces (1909) enters into the determination of penetration or nonpenetration of bacteria through the mucosa. This hypothesis certainly deserves experimental investigation.

The demonstration that cutaneous chilling causes reflex vasoconstriction and ischemia in the nasal cavity, nasopharynx, oropharynx, tonsils and palate at least furnishes a new and correct point of departure for future investigation, to replace the former false assumption of congestion.

That the mechanism we have elucidated is the only one concerned in exciting the acute cold it would be obvious folly to believe. The conditions determining the equilibrium between host and parasite in the tonsillar crypts and folds of the pharyngeal mucosa must be so extremely complex that many different factors are capable of disturbing it either in the direction of proliferation of the parasites and infection or of their annihilation.

A mechanism perhaps more often responsible than ours for the acute "cold" has been elucidated by Leonard Hill and F. F. Muecke (1913). These workers found that in hot moist crowded rooms, such as ill-ventilated theatres or meeting halls, the mucous membranes over the turbinate bones and nasal septum swell, become turgid with blood and tissue lymph, and covered with a thick secretion. In such crowded places massive droplet infection is likely to occur. On going out into cold, moist, outer air, the blood vessels constrict and the nasal mucous membrane is chilled but remains swollen with tissue lymph. These authors believe—and certainly with apparent justification—that this latter condition of the nasal mucous membrane affords a suitable condition for bacterial proliferation. They find that the dangerous primary passive congestion is much less if the air in the room is kept circulating and is not overheated.

The monograph of Leonard Hill (1919) especially urges the importance in causing colds of *contagion* and of the unnatural conditions of *indoor life*, where bodily vigor is not maintained by adequate outdoor exercise, crowding favors massive infection from those already actively infected and from carriers, and overheating of the atmosphere at head level causes passive congestion of the nasal mucous membrane and predisposes to infection. In Hill's monograph is to be found detailed discussion also of the effects on the respiratory membrane of altered respiration and of changes in temperature of the inspired air, as distinguished from the phenomena we have been especially studying, namely, the reflex effects on the mucous membrane circulation of chilling the body surface without altering the quality of the inspired air. The effect of physical exertion (without excessive fatigue, of course) in increasing respiration and hence transudation of liquid through the respiratory membrane, is emphasized by Hill

as a valuable factor in the prevention of initiation of infection of the respiratory membrane.

It should be clearly borne in mind that the factors of *contagion* and of *general bodily health* are probably of considerably greater importance to preventive medicine and the welfare of the community than the effects of chilling of the body surface.

We would reiterate also in order to minimize any possibility of misunderstanding. We are concerned in our experiments with *excessive* chilling of the body surface, which like overdosage of a useful drug, we believe may have ill effects. Certainly we would not encourage the unreasoning fear of drafts and exposure so often encountered. Good ventilation and circulating air in buildings, cold weather, and out-of-door living are needed for vigorous health; many people are unquestionably benefited by cold bathing. But excesses in this direction should also be avoided.

We are now in a position to outline an explanation for the annual cold-weather increase in incidence of upper respiratory diseases, which, although admittedly incomplete and in part speculative, would yet seem to fit well the known facts. With the beginning of cold weather, cessation of summer and autumnal out-of-door life, and beginning of hot air and steam heating, conditions of living for the average American and European become less favorable to general health and resistance. Opportunities for excitation of autoinfection by prolonged or excessive chilling become more frequent, as do those for cross-infection in crowded cars and meeting places, where mucous membranes are rendered more susceptible to infection by the close, hot atmosphere (Hill and Muecke, 1913). As the bacteria gain foothold and multiply on one after another susceptible mucous membrane it is in harmony with known immunologic facts to suppose that they tend to increase in virulence and so add another factor to the general tendency to increased prevalence of respiratory infection. With moderation of the weather and return to out-of-door life in the spring, aided possibly by some as yet unknown cyclic or other change in the bacterial invaders, we would expect the annual decline in prevalence. (Jour. Am. Med. Assn., 1920, lxxv, 1500.)

How practically to escape respiratory infection in so far as possible? Lead a vigorous and healthful life with adequate sleep, food, exercise and fresh air. Bathe daily. Keep the houses sufficiently warm to be comfortable, but not, as most American houses are in winter, overhot and overdry. Avoid contagion from infected persons, remembering that respiratory diseases are communicated chiefly through droplets of mucus sprayed into the air through coughing, sneezing or speaking. Avoid excessive irritation of the mucous membranes by tobacco smoke or other vapors. Avoid prolonged or excessive chilling. Avoid crowding in hot atmospheres. Obstructions to breathing or foci of infection if present should of course be removed. Some degree of "hardening" to exposure, graded and adapted to the needs of the individual, we believe is possible, and of the greatest service. As one valuable factor in this process, one of us at least after some eight years trial is convinced of the efficacy in his own case of a daily morning shower bath with warm water concluded with a few minutes of very cold water.

## IV. ANAPHYLACTIC "COLDS"

The discussions of vasomotor rhinitis and of asthma in the majority of the current texts will have to be entirely rewritten. The rescue of these affections from the nebulous province of diatheses and neuroses, their establishment upon a definite and sound, if not wholly complete, etiologic and therapeutic basis, makes up one of the chapters of modern experimental medicine upon which one's imagination lingers with most satisfaction. Since, however, thoroughly modern and adequate treatments of this subject are available (v. Goodale, 1918, and Walker, 1919) they will not be further discussed here beyond pointing out a subgroup of cases recently come within the experience of workers in the field, which simulate recurrent common acute colds.

I cannot do better than quote briefly from Dr. Walker (1919, p. 146):

"It is not at all uncommon for some patients to complain of what they call hay fever symptoms or very frequent head colds throughout the year. Since this kind of case seeks aid from the nose specialist, who terms the condition vasomotor rhinitis, the internist sees little of the condition, and even when the internist does see the case first he immediately refers it to the nose specialist. In the future, however, such cases should be tested for sensitization to proteins. Not infrequently are the emanations of animals the cause of these all the year hay fever symptoms. Horse hair dandruff and cat hair are frequently the cause of these spasmodic attacks of hay fever, which last a few minutes to a few hours or even a day or two, and in such instances the patient gives a positive skin test with the proteins of the hair. Less often the injection of various foods causes spasmodic attacks of short duration simulating hay fever. True seasonal pollen cases frequently complain of frequent head colds throughout the year and these head colds closely simulate short attacks of hay fever. In some of these pollen cases the sensitization serves to permanently render the mucous membranes extremely sensitive and irritable, so that sudden temperature changes, drafts, odors, and dust particles, are sufficient to produce symptoms, in other pollen cases bacteria seem to be the cause."

See also Goodale (1916), Floyd (1920), and Walker (1920). The important point here for the laryngologist, internist and pediatricist to remember is that frequently recurring "colds" may have protein sensitization as their basis and to be in readiness to do the skin tests when indicated.

## V. ACUTE INFLAMMATION DUE TO SYSTEMIC, TOXIC AND NEUROTIC FACTORS, TO MECHANICAL AND CHEMICAL IRRITATION, TO EXTREME LOW TEMPERATURES, AND TO NASAL OBSTRUCTION

A discussion of the etiology of acute inflammation of the nose, throat and tonsils should at least give passing mention to various inflammatory conditions of the upper respiratory tract associated with more general affections. Acute rhinitis occurs as a local prodromal symptom of influenza, measles, scarlatina, whooping cough, enteric typhus, small-pox, chicken-pox, secondary or congenital syphilis and glanders. (Thomson, 1913, p. 113.) Similarly for acute pharyngitis. Diphtheritic and gonorrheal rhinitis occur; also that of erysipelas.

Acute upper respiratory inflammations may be set up in those exposed to local irritants, as millers, furriers, sawyers, tobacco-workers, ivory and steel turners, and decorators, or (Schade, 1920) when the mucous membranes are exposed to extreme low temperatures. Locally the vapors of formalin, the halogens, ammonia and the fuming mineral acids and dust and coal and tobacco

smoke may give rise to them. A destructive form of rhinitis may occur in workers in bichromate of potash, mercury, arsenic or osmic acid. Acute pharyngitis not infrequently follows traumatism, as the swallowing of hot fluids, corrosives, hot condiments, raw spirits, and the impaction of foreign bodies. It may be induced by operations on the pharynx with much sponging or traction on the tissues, e.g., emucleation or morcellment of the tonsils. Secondary infection may, of course, aggravate any such primary condition.

Acute mucous membrane inflammation may occur in connection with the administration of certain drugs, as potassium iodide, arsenic, mercury and antimony.

Acute rhinitis "is predisposed to by all obstructive affections of the nose and postnasal space. Chronic hypertrophic rhinitis, deformities of the septum, polypi and chronic empyemata conduce to the contraction and exacerbaton of acute nasal catarrh; and frequent and persistent rhinitis in children is generally due to nasopharyngeal adenoids. Indeed, a large majority of children who are reported to be always catching a 'cold in the head' will be found to possess inflamed or overgrown pharyngeal tonsils." (Thomson, *ibid.*)

A vasomotor turgescence of the nasal mucous membrane may originate in certain individuals from some reflex, such as chilling of the feet, sudden exposure to bright light or damp air, inhalation of vitiated air, gastric disorder, or sexual irritation.

#### VI. CONCLUSIONS

The following causes of acute inflammation of the pharynx, tonsils and nose are recognized in the present dissertation:

1. The filterable virus of Kruse and Foster, inducing apparently a clinical entity, a type of acute coryza. According to the experiments of its discoverers, this is of relatively high virulence and may cause infection practically independently of the action of exciting factors.

2. Various bacteria, including the *Pneumococcus*, *Streptococci*, *B. rhinitis*, *B. diphtheriae*, Friedländer's bacillus, *B. influenzae*, and probably also *M. catarrhalis*, *B. septus*, *M. paratetragenus*, *S. aureus*, and possibly others seem to be capable of inducing infection of variable extent, duration and symptomatology. The relative virulence of the microorganisms also varies within wide limits—both between themselves and from time to time in the same organism—in some instances high, infections of epidemic proportions, largely independent of exciting factors, may be produced; in other instances low, sporadic infection may occur only when some factor or factors serve to depress resistance, general or local, to the point of vulnerability.

3. Protein sensitization, the basis of vasomotor rhinitis and of true bronchial asthma, the underlying cause also of a relatively infrequent sub-group of acute recurrent "colds."

4. Various systemic diseases, drugs, mechanical and chemical irritants, chronic nasal affections and reflex neuroses.

One factor by which resistance to bacterial infection may be lowered is excessive chilling.

Experiments by many workers have shown that animals whose blood temperature is lowered may have decreased resistance to infection. This, however,

is probably not the mechanism by which chilling excites the common upper respiratory infections in man.

Experiments have shown that chilling of the body surface of animals causes congestion of many internal organs. Reasoning from a faulty analogy, the theory has been evolved and made widely current that similar congestion occurs in the upper respiratory mucous membranes of man when chilled, and is responsible for the local lowering of resistance. However the work of the present authors has shown that the opposite is true, namely, that chilling of the body surface causes reflex vasoconstriction and ischemia in the mucous membranes of the nasal cavity, postnasal space, and palate, oropharynx, nasopharynx and palatine tonsils. It seems not improbable that the ischemia may be the means of lowering local resistance. In other instances the mechanism of Hill and Muecke, i. e., crowding in overheated places followed by emergence into a cold atmosphere, is doubtless responsible for colds.

During the course of the experiments by which the vasoconstrictor reaction to chilling has been demonstrated, some ten cases of cold or sore throat, usually mild, have appeared among the subjects. In a number of instances the clinical symptoms were accompanied by interesting bacterial changes.

It is a pleasure to thank the friends without whose generous aid as subjects much of the present work would not have been possible.

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## THE RELATION OF PATHOLOGISTS TO THE INSTITUTIONAL PRACTICE OF MEDICINE\*

BY WM. CARPENTER MACCARTY, M.D., MAYO CLINIC, ROCHESTER, MINNESOTA

THE paper is presented to this body of scientists as one of a series which has the following objects:

1. To show pathologists (including bacteriologists, serologists, immunologists, and other workers in pathology) the economic part which they should play in the practical consideration of patients.
2. To show clinicians that laboratory investigators can, and sometimes do, play an important rôle in the practice of medicine.
3. To assist in bridging the gap which now exists between what has been called pure science and its practical application to patients.
4. To suggest the great probability that some of the most important fields of scientific research may be found in direct connection with the study of patients.
5. To relieve the minds of young aspirants in research from the conception that the most fundamental research must of necessity be carried on in isolated research institutions.

The following facts have been the stimuli for this series of reports:

1. Intimate, valuable correlation of laboratory and clinical facts and methods is being practiced in some large medical and surgical clinics.
2. Such correlation is unfortunately rare on a large and significant scale in most large and small hospitals throughout the country.
3. There is a demand for such correlation because the writer weekly receives letters from hospitals requesting aid.
4. There is a great dearth of pathologists to fill these positions, a fact which is leading practical clinicians to have girls trained to do laboratory work under their own imperfect direction.
5. The writer has seen much inefficient laboratory work by poorly and inefficiently trained girls and interns. The work of many such poorly trained workers discredits a science which you and I desire to see maintain its high place of true usefulness.
6. Many professors of pathology throughout this country manifest a lack of interest and an abundance of indifference in the practical application of their science to clinical practice.

Table I shows what is being done by one institution in the application of laboratory methods to the practice of medicine.

It may be seen that each patient averaged 3.72 laboratory reports, each of which usually entails many examinations or tests.

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\*Presented before the American Association of Bacteriologists and Pathologists, New York, April, 1920.

TABLE I  
LABORATORY EXAMINATIONS OF 60,645 PATIENTS

Determinations of the basal metabolic rate .....	5,650
Gastric analyses .....	11,946
Other clinical laboratory tests .....	192,739
Electrocardiographic records .....	2,021
Fresh tissue examinations .....	12,964
Neeropsies .....	465
Laboratory reports .....	225,785

Ninety-nine and two-tenths per cent of the patients were alive when the examinations were made; examinations were made on 0.8 per cent at neeropsy, and 97.7 per cent of the patients were living at the time the reports were made.

These data actually show what the active pathologist can do and is doing in the practice of medicine. It would be impossible, at present, to state what percentage of patients was actually benefited by these examinations, but it may be stated accurately that all reports had positive or negative value in establishing a diagnosis and that the diagnosis governs the therapeutic measures. The functions of the pathologists who were responsible for the 225,785 reports may be divided as follows:

1. Confirmation of diagnoses, of which the clinician might have had some suspicion.
2. The diagnosis of conditions which the clinician did not suspect.
3. The recognition of accessory pathologic conditions.
4. The correction of clinical diagnoses.
5. The confirmation of positive clinical diagnoses.
6. The determination of stages in the course of disease.
7. The determination of the physical status of the patient preparatory to possible operation or other treatment.
8. Assistance in determining the extent of operations.
9. Determination of data for preoperative, operative and postoperative prognosis.
10. Determination of the cause of death in nonsurgical and in surgical cases.
11. Determination of the cause of death due to incorrect medical or surgical judgment.
12. Determination of the cause of death resulting from incorrect operative technique.
13. Assistance in determining causes and modes of surgical infections.
14. Assistance in clinical, surgical, and laboratory research.
15. The actual treatment of disease.

I shall not attempt to analyze the relation to the patient of metabolic rates, gastric analyses, electrocardiographic records, bacteriologic and serologic examinations herewith presented. This must be left for some future time. But there is one field in pathology worthy of consideration in detail and one with which I am more intimately familiar, that is, the field of tissue pathology. Most pathologists study material at neeropsy and hence see end



results; some have occasion to examine tissue from living patients for diagnostic purposes. It is this group of pathologists in whom I am at present especially interested because most of them are not pursuing their vocation to the fullest extent of their practical capacity. They are pathologists but they are not clinicians practicing pathology as a specialty in association with patients. This field of possible activity of such pathologists is illustrated in Table II.

TABLE II  
SPECIMENS OF TISSUE EXAMINED DURING 24,368 OPERATIONS

	SPECIMENS	DIAGNOSIS POSSIBLE ONLY WITH A MICROSCOPE
HOSPITAL A (GENERAL SURGERY)		
Breast	289	18 ( 6.2 per cent)
Extremities	118	20 (16.0 per cent)
Gall bladder	1,086	0
Genitourinary organs	486	33 ( 6.7 per cent)
Head	33	17 (51.0 per cent)
Intestines	249	32 (12.0 per cent)
Lip and glands	44	6 (13.0 per cent)
Neck	259	54 (20.0 per cent)
Stomach	191	61 (31.0 per cent)
Thyroid	1,638	4
Trunk	153	31 (20.0 per cent)
Uterus (portion)	165	11 ( 6.0 per cent)
Uterus (entire)	655	21 ( 3.0 per cent)
Tubes	625	4
Ovaries	601	5
Parovarian cysts	75	0
Specimens for diagnosis	432	207 (47.0 per cent)
HOSPITAL B (GENERAL SURGERY)		
General specimens	2,635	461 (21.0 per cent)
HOSPITAL C (EYE, NOSE, AND THROAT)		
Diagnostic specimens	461	432 (93.0 per cent)
CLINICAL OFFICES		
Diagnostic specimens	586	558 (95.0 per cent)

Twelve thousand nine hundred sixty-four fresh tissue examinations showed that 21.3 per cent of all registered patients (60,645) presented surgical specimens. More than 40 per cent of all the registered patients came to operation and more than 57 per cent of all patients operated on furnished pathologic specimens.

Diagnosis of many pathologic conditions can be made before operation; some can be diagnosed during surgical exploration before tissues are removed; and still more can be diagnosed grossly after tissues are dissected and removed; but there is a certain percentage which cannot be diagnosed positively grossly and must be submitted to microscopic examination. This method of diagnosis was of necessity applied to 15.2 per cent of all specimens in the series, although the percentage varies from 15 per cent to 25 per cent from year to year. The total percentage not only varies but the percentage on different organs and tissues varies from year to year. However, in a series of records covering

30,130 specimens from all sources, 21 per cent were of necessity examined microscopically.

The real value to the patient cannot be seen from this total percentage, but may best be seen from examining the character of certain special examinations. Thus it may be seen that 2,279 specimens (series of 800, 432, 586, and 461, respectively) were removed for diagnostic purposes, which means that 3.7 per cent of all registered patients received one form of direct preoperative and operative aid from the tissue pathologist. While this is perhaps the most important function from the standpoint of the patient, the act of picking up incorrect clinical and surgical diagnoses during operation is of just as great importance. This function will be considered in a future paper. Sufficient data are already at hand to justify the statement that incomplete operations and incorrect prognoses are avoided by virtue of the activity of a clinical pathologist working in conjunction with appreciative surgeons who lay no claim to being either great macroscopic or microscopic diagnosticians. The clinician's diagnostic efficiency has already been described in another article of this series. It was seen in a comparative study of clinical preoperative instructions and pathologic findings that the clinician is correct in 95 per cent of cases when he makes positive diagnoses, but experience has taught him that positive diagnoses are frequently dangerous, detrimental to his reputation, and productive of injustice to the patient. While this percentage is high, it has been pointed out, for example, that 22 per cent of all carcinomas of the breast seen in the surgical laboratory are actually discovered by the pathologist and that when the clinician makes a questionable diagnosis of a malignant condition in the breast he is correct in 49 per cent of the cases in so far as malignancy is concerned. Thus it may be seen that the tissue pathologist has a great function which he is capable of exercising, and that microscopy is just as essential to clinical diagnosis, in practice, as in auscultation, palpation, and other methods of physical examination. It is not only an accessory, it is an essential. Therefore, the expert microscopist is an essential factor in clinical diagnosis.

# LABORATORY METHODS

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## THE DIAGNOSIS OF TYPHOID AND PARATYPHOID INFECTIONS\*

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BY HENRY J. GOECKEL, PHM.D., CRANFORD, N. J.

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IN a previous paper<sup>1</sup> attention was called to the early diagnosis of typhoid and paratyphoid infections by the identification of the bacteria in the urine with high titer agglutinating serum. The agglutinating method was first used in an attempt to determine if motile rod bacteria, frequently encountered in urine with pus, were of the typhoid-colon group, without resorting to cultural methods. The diagnostic value soon became apparent. Since the last publication the method has been applied successfully in ways not anticipated at that time.

The cases previously cited indicate that the blood count if relied on to verify the physical diagnosis is at times very misleading, especially when the Widal reaction is negative.

The classic Widal reaction is today a questionable test on which to rest a diagnosis of typhoid infection, as is done by many physicians. Beside the late appearance of a positive blood test in many cases, it has in recent years been demonstrated that homologous agglutinins not only exist for all the members of the typhoid-colon group of bacteria, but likewise for these and for gonococci.

We must also take into consideration that there are probably ten million people in the United States who either through typhoid infection, or by prophylactic immunization have developed or been sensitized to agglutinin production. This agglutinin production persists over long periods of time. Consequently we are liable to get a positive Widal reaction in low dilutions of serum where an active typhoid infection is not present, thereby possibly misleading rather than aiding in the diagnosis.

It has likewise still to be shown whether a gonococcal infection can reactivate or bring about an increased production of the homologous agglutinins in a typhoid-paratyphoid sensitized person when a gonococcal infection exists. This is being investigated by one of our staff.

In the dilutions of the high titer agglutinating serum employed no cross fixation is obtained.<sup>2</sup> This has been demonstrated on pure cultures and various mixtures of the same by the writer.

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<sup>1</sup>Goeckel, H. J.: Jour. Lab. and Clin. Med., January, 1920, v, No. 4.

<sup>2</sup>Titer of serum employed was typhoid, 1:5000; paratyphoid A and B, each 1:10,000; B. coli, 1:2000. These were used respectively in 1:2500, 1:5000 and 1:1000 dilutions.

I have recently employed this method on the bacteria of fresh feces from suspected cases. A selected particle of the feces was suspended in physiologic salt solution, thoroughly agitated to break up all clumps. Solid material was allowed to settle and the bacterial suspension was diluted to the usual turbidity employed for the hanging-drop Widal test.

In one of the two feces examined a recognizable clumping and inhibition of motility for typhoid bacilli was given, and in the other for paratyphoid, type B. A dense clumping was secured in each case with the bacillus coli agglutinating serum and none with the paratyphoid, type A, serum. This application apparently affords another ready means of recognizing the presence of such bacteria in case no blood or urinary bacteriemia is found at the time of examination. It likewise affords a means for promptly passing on the bacterial content of the feces in convalescing cases, making many of the bothersome cultural examinations unnecessary.

The following case history shows that a typhoid bacteriemia can be found in the circulating blood and in the urine at the same time in an acute typhoid infection:

Child, L. M., (Dr. W. M.) May 27, 1919, aged sixteen months. Throat swab and culture show a few staphylococci.

*Blood*.—Hemoglobin 69 per cent, index 0.622; red cell count 5,580,000; white cell count 4,800, polymorphonuclear leucocytes 43 per cent; eosinophiles 2 per cent, azurephiles 1 per cent; lymphocytes 40 per cent (large 30 per cent, small 10 per cent). Red cells normal except for hemoglobin content. Noticeable number of gram-negative rod bacteria present on the blood slides.

*Urine*.—Acid, distinct trace of albumin, distinct acetone test, moderate number of slightly clumped leucocytes and considerable gram-negative rods which type for bacillus typhosus.

In the following case the urinary bacteriemia was the only definitely confirmatory finding.

Child E. A., (Dr. J. V.), Aug. 26, 1920, age nine years. Persistent temperature, otherwise no physical symptoms of note. Child robust, bright and apparently comfortable. Typhoid suspected. Three experienced physicians made a thorough physical examination and could detect nothing of note. Three negative Widal tests reported by the state laboratory.

Aug. 26, 1920. *Blood*.—Red cell count 4,740,000; white cell count 11,600; polymorphonuclear leucocytes 63 per cent, mononuclear leucocytes 14 per cent, lymphocytes 23 per cent (large 10 per cent, small 13 per cent); no plasmodia.

*Urine* showed considerable pus and motile rod bacteria, which type for bacillus paratyphosus, type B.

The following case was an unsuspected typhoid infection:

Mrs. B. M., (Dr. F. H.) April 9, 1920, Cystitis. Frequency in urinating, discomfort in pelvic region. Passed blood in the urine. Had typhoid in 1907 and "almost died from it."

April 9, 1920. *Urine*.—Catheterized, considerable pus, no tubercle bacilli.

April 12, 1920. *Urine*.—Catheterized, considerable pus, no tubercle bacilli, but numerous motile rods which type for bacillus typhosus. This specimen had been placed in the incubator overnight.

*April 20, 1920. Urine.*—23 hour lot—sp. gr. 1.020, trace of albumen, urea 1.7 per cent, squamous epithelium, considerable pus, and bacillus typhosus, no tubercle bacilli.

*May 25, 1920. Urine.*—Sp. gr. 1.036, no albumin, urea 2 per cent, no pus, etc., urine culture—negative.

*June 4, 1920.* Widal reaction on blood was negative.

The following case was an emergency operation which gave no typhoid history:

Mrs. G. (Dr. P. Z.) March 13, 1920, aged twenty-five years, admitted for ruptured ectopic pregnancy which showed a rupture in uterine half of the left tube. Abdomen filled with free blood and clot.

Patient had always been in good health. One and a half years previous had an early (2 or 3 month) abortion. Two days before admission was taken with sudden violent pain in abdomen, accompanied by great shock and deathly palor. Morphine relieved pain. Another attack on morning of admission, was extremely weak, P. 144, R. 124, T. 101°F.

Before operation and after an intravenous injection of 700 c.c. of saline the hemoglobin was 60 per cent and the urine showed a sp. gr. 1.030, very faint trace of albumin, small amount of pns and a moderate number of uric acid crystals. Immediate recovery from operation was satisfactory, P. 120-144, T. 99.5 to 101°F.

*March 13 and 14.* Patient vomited a good deal, March 14 quite tympanitic but no pain.

*March 15, 1920.* A. M., slightly irrational; 9 to 10 P. M., irrational, talks incessantly.

*March 16, 1920.* Same, not much relieved by medication, etc. Gas and feces escaping freely.

*March 17, 1920. Urine,* sp. gr. 1.020, reaction neutral, faint trace of albumin, trace of acetone, few casts, considerable pus.

*March 18, 1920. Urine.*—Trace of albumin and of acetone, considerable pus and many motile rod bacteria.

*March 19, 1920.* Wildly delirious, very restless, screams. Urine, especially obtained at 3 P. M., strong albumin and strong acetone tests. Moderate number of hyaline and few granular casts, and numerous pus cells. The specimen was turbid throughout with motile rod bacteria. Upon standing these still formed a film throughout two-thirds of the specimen. They were identified by agglutinins as bacillus paratyphosus, type B. Patient died at 9 P. M., complete autopsy refused. Exploration of the operative area showed no peritonitis or wound infection. Death due apparently to a paratyphoid toxemia.

#### SUMMARY

The high titer agglutinating serum can be employed to demonstrate the presence of typhoid and paratyphoid bacilli in feces both for diagnostic purposes in suspected typhoid infections and to determine their presence in the feces from convalescing cases without resorting to cultures.

The typhoid bacilli can appear in the urine at the same time that an extensive circulatory bacteriemia exists, before a positive Widal reaction is obtainable. The finding of the bacilli in the urine may prove the only definite means of establishing a definite typhoid diagnosis.

A typhoid bacillus cystitis can apparently exist without producing an agglutinin production in the blood.

A typhoid or paratyphoid bacteriemia unsuspected in an operative case may prove disastrous to the patient. This paper and the previous one show that the detection and identification of motile bacilli in urine can prove of considerable diagnostic value.

# THE ROUTINE DETERMINATION OF CREATININE AND ACETONE IN URINE\*

BY HENRY J. GOECKEL, PH.D., CRANFORD, N. J.

NEVER having noted the inclusion of a creatinine determination in the usual routine clinical analysis of urine by others, the following modification of a recognized test as used by the writer may be of value. It gives a roughly quantitative creatinine determination without much additional labor and requires no special apparatus.

## REAGENTS

1. A saturated aqueous solution of sodium nitroprusside. This keeps for a week or more when placed in an amber glass bottle. 2. A sodium hydroxide solution. 3. Glacial acetic acid.

To a row of test tubes, approximately 5 c.c. (mils) of each urine is added in consecutive order. Ten drops of the nitroprusside solution are then added to each tube followed by sufficient sodium hydroxide solution (a few drops) to produce the maximum intensity of red color.

A dense cherry red color indicates a normal or high creatinine content, while a paler shade will indicate a low content. This must be noted at once as the color begins to fade in the course of a few minutes. By examining a series of urines they can be readily contrasted. Those which appear low in creatinine content are noted.

Glacial acetic acid is then added to each tube to determine acetone. In the absence of acetone, when the creatinine is normal or low, the urine returns to approximately its normal color. Should the creatinine be high, various shades of green will become evident. When the creatinine content is very high a deep blue precipitate will form.

The presence of acetone will be indicated by the usual production of various shades of red when the glacial acetic acid is added.

This application of the acetone test to indicate creatinine affords a ready means to differentiate between those nephritic cases showing probable creatinine retention and those which are not so far advanced. It also affords a ready means in conjunction with the other routine tests for detecting surgical cases liable to be very poor risks, due to advanced nephritic conditions. It is of service in hospitals not fortunate enough to have the proper facilities and technician service to perform the more extended and reliable quantitative blood tests.

I use this test as the principal factor for advising against an operation when the creatinine is low. On the other hand I have yet to note a case showing a slight albuminuria and few or no casts, but with a normal or high creatinine content which did not pass through the anesthesia and operation uneventfully as related to the nephritis. I have seen many such cases which returned to normal following operation.

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## BENIGN TUMORS OF THE GASTROINTESTINAL TRACT\*

By EDWARD E. H. BOYER, PH.D., COLUMBUS, O.

THE finding of benign tumors in the gastrointestinal tract is quite unusual, and frequently very interesting and important. The least important of such neoplasms are probably those in the stomach, since the chance for obstruction to the canal is less here than in other portions of the gut. The small intestine, especially the ileum, appears to be the seat of benign growths more frequently than any other part of the alimentary tract; and the appendix gives rise to tumors less frequently than any other part. The principal types of benign neoplasms are, in order of frequency, myomata, lipomata, adenomata, fibromata.

Sometimes such growths give rise to no symptoms, and are found by accident during operations for other conditions, or at autopsy. In some cases the symptoms are very indefinite and misleading, especially if the tumor is growing toward or beyond the serosa. But when definite symptoms appear the patient usually complains of obstinate constipation, less often of intestinal hemorrhage. The constipation in such cases may be due to intussusception, but is more likely to be caused by obstruction of the lumen due to the presence of the tumor which has grown toward or beyond the mucosa. A diagnosis probably has never been made.

Sometimes the tumors grow into and obstruct the lumen; but others may progress outward to the serosa. Those of the former case, obviously, are the ones which cause most pain and discomfort, and are usually more dangerous to the patient.

Among the factors which may be responsible for the direction of growth of the neoplasm are (1) point of origin of the new growth, (2) mechanical resistance of adjacent layers, (3) biologic resistance of neighboring cells, (4) intestinal stasis.

The symptoms may simulate those of malignancy in the affected part, so that the prognostic value of these neoplasms should not be overlooked. The following two case reports are appended:

CASE 1.—*Service of Dr. Brock.* A farmer, aged sixty-five years, was admitted to Mt. Carmel Hospital on account of pain about the umbilicus. There was no history of injury to the abdomen. The patient had had "stomach trouble and indigestion" for years, but was seldom constipated until two years ago, when violent catharsis was used. The patient has not defecated for five days. There has been considerable pain in the epigastrium, with abdominal distention. Patient has vomited stercoraceous material several times. Physical examination reveals nothing of further importance. At operation a tumor of the ileum was removed. An end to end anastomosis was performed.

*Pathologic Report.*—The piece of intestine measures 8 cm. in length by 2½ cm. in diameter. In the central portion there is a moderate bulging. On section one finds the lumen obstructed by a firm nodule which is pedunculated to the intestinal wall. This nodule measures 2 cm. in diameter. It is covered with mucous epithelium, and is circumscribed but not encapsulated. On section the cut surface presents the appearance of muscle tissue.

\*From the Pathological Laboratory of Mt. Carmel Hospital, Columbus, O.

Grossly the muscle fibres appear to originate in the muscular layer of the gut. Histologically one finds the tumor covered with a layer of simple columnar epithelium which has not atrophied. The muscle fibres run in all directions, sometimes wavy, sometimes in straight bundles, and occasionally in whorls. There is a moderate amount of connective tissue stroma. Capillaries are few, and round cell infiltration is absent. The gut, on both sides of the point of origin of the neoplasm, is of normal appearance. Just below the pedicle, however, the muscular layer thickens, loses its normal orientation, and grows toward the mucosa. The new growth is readily seen to have pushed the mucosa before it, not penetrated through it, as it grew into the lumen of the intestine. The connective tissue of the mucosa and the muscularis mucosae are not included in the covering of the tumor; only the epithelial layer is left. It is probable that the connective tissue and muscle cells became atrophied by pressure, and only the epithelial cells were able to regenerate rapidly enough to keep pace with the progressive growth of the tumor. The structure of the tissues is very beautifully demonstrated by using Van Gieson's picrofuchsin stain.

*Diagnosis.*—Leiomyoma.

CASE 2.—Female, aged fifty-two years. Complained of pain in the pelvic region. At various times she complained, also, of digestive disturbance with some gastric pain. A clinical diagnosis of ovarian cyst was made. At operation an ovarian cyst was found and removed. During exploration of other abdominal organs a mass was found, by palpation, on the greater curvature of the stomach, about three inches above the pylorus. It seemed to penetrate the entire wall of the stomach. Section was made, and the tumor was removed in one mass. The remaining parts of the viscera were apparently normal. The patient made an uninterrupted recovery.

*Pathologic Report.*—The mass measures  $3\frac{1}{2} \times 2\frac{1}{2} \times 2\frac{1}{2}$  cm. The peritoneal surface is smooth, of light color, and discloses occasional blood vessels. It was pedunculated, the pedicle measuring  $\frac{1}{2} \times \frac{1}{4}$  cm. On section one finds a mass of firm, fibrous tissue involving the entire specimen. The tumor is encapsulated, the capsule measuring  $\frac{1}{2}$  mm. in thickness. The cut surface exhibits a pearly translucent appearance. The cells appear to be arranged in wavy bundles and in whorls, and there is a resemblance to the cut surface of certain uterine myomata. Microscopically one finds many fibroblasts evenly distributed throughout the neoplasm. The collagen fibrils are quite numerous, and a few fibroglia fibrils are also found. The cells are grouped in bundles which lack definite orientation and are in many different planes and directions. Blood capillaries are not especially numerous, and are evenly distributed. The endothelial lining is prominent in some vessels, the individual cells appearing swollen. In other areas the endothelial cells appear flattened. There is a diffuse lymphoid cell infiltration which is not particularly marked except in some perivascular regions. A few plasma cells are mixed in with the other cells. There are no areas of hemorrhage, and degenerative changes cannot be found. One looks in vain for muscle and epithelial cells.

*Diagnosis.*—Fibroma of the stomach.

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\*This case was from the service of Dr. Shumaker, Dover, O.



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## EDITORIALS

### *The Route of Absorption of Inhaled Substances*

SOME months ago the valuable work of Blake and Cecil on experimental pneumonia was reviewed in these pages. These investigators produced typical lobar pneumonia in monkeys by injecting broth cultures of pneumococci into the trachea; they also produced bronchitis and bronchopneumonia by similar injections of influenza bacilli of exalted virulence. They concluded from studies of an animal killed three hours after pneumococcus inoculation, that primary tissue invasion took place through the walls of the larger bronchi near the root of the lung, rather than through the alveolar walls. Pneumococci were most numerous, and the beginning tissue changes most pronounced, in the peribronchial tissues around the hilum. They do not deny the possibility of invasion through the alveoli, but feel that the objective findings did not support this view.

Winternitz, Smith, and Robinson have called attention to a possible error or complicating factor in the interpretation of such experiments, when the technic is such as to involve trauma, especially to the submucosa of the trachea. Apparently this possibility cannot be excluded when such trauma exists. Moreover, it must be recognized that pneumococci, which are capable

of producing lesion of the bronchial mucosa, may invade it readily, while other organisms and substances fail to do so.

The experiments of Mullin and Ryder, carried out last year in our research laboratory at Colorado Springs, and recently published, seem to provide examples of invasion which is chiefly, if not entirely, through the alveolar epithelium. They allowed rabbits to inhale suspensions of finely divided carbon (India ink) and of virulent human tubercle bacilli. These fluids were dropped into the nasal fossae with a pipette, and allowed to run back into the pharynx, whence they are inhaled, owing to the rapid breathing and inefficient laryngeal control of these animals.

Two rabbits received an emulsion of tubercle bacilli. They were killed after seven weeks, and both showed massive tuberculous infiltration of the upper and middle lobes of the right lung, with moderately advanced tuberculosis of the bronchial nodes. The cervical nodes, spleen, and mesenteric nodes were all negative both grossly and microscopically. The parenchymatous character of the lesions, and the involvement, in both cases, of the whole of the apical and middle lobes of one lung, without other visible lesions, favors a primary alveolar process rather than a peribronchial one, but of course is by no means conclusive.

The evidence from the rabbits that were given India ink is more impressive. Free ink was found all along the respiratory tract. In every instance there was very extensive blackening of the parenchyma of the lung, with moderate to marked discoloration of the bronchial nodes. There were slight to considerable amounts of carbon in the spleen and traces in the kidney. In no case was any found in the mesenteric nodes. The aspect of the lungs was very striking, some lobes being entirely black, others showing clusters of large black blotches, while others were hardly discolored at all. "The appearance of all this pigmentation, its overwhelming quantity, is absolutely distinct from spontaneous anthracosis, which in rabbits is seldom grossly visible in the lungs, and if so occurs only as fine stippling, never as masses or blotches."

In order to exclude all possibility of absorption from the digestive tract, "Rabbit 4 was given about 3 c.c. of India ink at one time and killed half an hour later. Autopsy showed much ink in the nose, mouth, pharynx, larynx, esophagus, trachea and bronchi. The stomach was filled with vegetable material and this was blackened over a radius of about 2 cm. around the esophageal opening, but there was no discoloration below this sharply defined level in the digestive tract, the remainder even of the stomach contents being free from ink. The lungs showed almost complete blackening of the left upper lobe, and marked blackening of the left lower and right middle lobes. The bronchial nodes showed marked hyperemia and a moderate amount of carbon scattered through them. The cervical and mesenteric nodes were negative. The presence of carbon all along the inhalation route, with its complete absence from the intestine, completes the proof that the lung findings are due to inhalation."

Microscopic sections of the lungs in several animals "showed carbon in the smaller bronchi and indicated that the blackening of the lungs was

lobular in arrangement, the alveoli being packed with carbon, much of which had been taken up by large phagocytic cells, evidently the alveolar cells of the lungs; and the deeper structural tissue of the lungs contained many of these cells apparently travelling along the lymphatics." "The behavior of these cells in this instance, as well as their proliferation in acute and tuberculous pneumonia, emphasizes their close relationship to the fixed endothelial cell and the large mononuclear leucocyte. They seem readily to engulf particles which arrive on their surface, and to proliferate under the stimulus, become detached, and either to be cast off in the expectoration or taken up and carried along the course of lymphatic absorption. The cells of the upper respiratory mucous membrane, on the other hand, do not appear to be phagocytic, but rather to throw off foreign particles by means of their secretion."

The authors here follow Sewell's interpretation as to the origin of the intraalveolar phagocytic cells. In view of the work of Foot on inhalation tuberculosis, it seems likely that these cells really arise from the endothelium of the capillaries in the alveolar walls, and migrate rapidly into the air spaces under the stimulus of foreign particles arriving there.

Foot, whose work complements that of McJunkin on endothelium, injected water-proof India ink intravenously to produce vital discoloration of all endothelial cells, both fixed (vascular and sinusoidal) and free (large mononuclears), and observed carbon-bearing cells in the capillaries of the alveolar walls, escaping into the air spaces, and lying free in the bronchi. These cells phagocytosed carmine granules and tubercle bacilli injected into the trachea. He says, "These cells . . . play the principal part in the reaction to the tubercle bacillus in the air-borne infection and are evidently the cells which Sewell thought to be epithelial." In inhalation tuberculosis he finds the first site of lesions in the alveoli. Desquamation of carbon-free alveolar cells and exudation of carbon-bearing endothelial cells occur together, and both types of cell may contain tubercle bacilli. The first formation of definite tubercles occurs almost simultaneously in the alveoli and in the interstitial tissue.

Similarly, Gardner finds that the alveoli first give evidence of lesion after inhalation of Type R1 tubercle bacilli, and of granite dust.

In the microscopic sections from the lungs in Mullin and Ryder's animals, we do not find carbon-laden cells in any quantity in the bronchial mucosa, as we should expect to do if these structures were readily capable of absorption, and this is true even when the animals were killed a short time after inhalation, when the small bronchi as well as the alveoli are seen to be full of carbon. So far as the evidence of these experiments goes, the normal bronchial mucous membrane apparently does not absorb. Possibly the peribronchial findings of Blake and Cecil may be due to the concentration of bacteria originally absorbed from the alveoli and converging down the lymphatics to the hilum, as described by Krause, rather than to absorption through the bronchial walls in the immediate vicinity.

Some recent observations of our own, not yet reported, may be worth mentioning here. In a study of certain phases of guinea pig anatomy and

their bearing on experimental tuberculosis, we injected India ink into the spleens of normal pigs. If the injection is massive enough, some of the ink reaches the lungs by way of the vena cava, heart, and pulmonary artery, and is found scattered through them in endothelial cells both fixed and free. These carbon-bearing cells are numerous in the alveolar walls, but very rare in the air spaces, and there is no proliferation or desquamation of alveolar epithelium. A good many of the pigmented cells are found along the peribronchial structures, the route, that is, of the vessels which bring the carbon to the lungs and of the lymphatics which take it up and carry it to the bronchial nodes, where it is found in considerable amount. Carbon-containing endothelial cells, both fixed and free, are found in the bronchial mucosa all the way from the trachea to the smallest bronchi, and many of them seem to be passing between the epithelial cells into the lumen, where they occur with moderate frequency. In Mullin and Ryder's animals, though the small bronchi were often choked with carbon and carbon-filled phagocytes, discolored cells were virtually absent from the bronchial mucosa.

In short, carbon particles reaching the free surface of the bronchial mucous membrane from the air do not appear to be absorbed; while on the contrary, carbon particles reaching this membrane by way of its circulation seem in part to be eliminated through it. The function of absorption is vested in the alveolar, not the bronchial, epithelium, yet the latter may be the site of primary lesion and early absorption in the case of a virus especially adapted to attack it.

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—G. B. W. (C. T. R.)

#### *Complications of the Arsphenamine Treatment of Syphilis*

ONE of the interesting aspects of syphilology is that which deals with the so-called organotropic action of the *Treponema pallidum*. It has been some years since the possibility was expressed that there were different types of treponemas which were responsible for different syphilitic manifestations, for instance tabes or other nervous forms of disease were due to one type, the neurotropic; hepatic manifestations were due to an hepatotropic type, and so on. The converse also is possible that the localization of spirochetal infection is not due so much to the organotropic action of the infecting organisms as to the organic susceptibility of the tissues of the body to the spirochetes. It is

possible that the reasons for organic localization of the organism causing syphilis are the same as those concerned in the localization of the streptococci of Rosenow. However that may be there is in the literature the account of the seven glass blowers, all of whom developed labial chancres from the same infected source. Five of these developed tabes and two general paresis. Also there are the cases of Morel-Lavallée—five men infected by the same woman. One of these developed syphilitic meningitis, two, general paresis, and one, a syphilitic psychosis. Fischl reports three cases of icterus which he believes give evidence of a hepatotropic variant of the spirochete. In one case, a woman, the jaundice followed a course of antisyphilitic treatment but disappeared under further treatment; in the second case, the husband of the first, jaundice developed and disappeared before treatment was commenced. The third case, one from which the husband had contracted the disease also had jaundice during treatment.

The main reason for separating such cases from other ones in which icterus presents itself lies of course in the common source of infection. Jaundice itself is not excessively rare.

Strathy, Smith and Hannah call attention to 47 cases of hepatic complications of treatment (jaundice, or atrophy) in 8 of which a fatal issue occurred. In such cases the onset of symptoms was seldom earlier than five weeks after cessation of arsenical treatment, and these symptoms are referred to the arsenic rather than to the benzol group of the drug used. French after calling attention to the fact that, in his experience, toxic jaundice is rare, expresses the belief that there are two types of jaundice in early syphilis; one which often occurs before arsenical treatment, and is syphilitic in nature and due to the action of *Tr. pallidum* on the liver cells; the second, which develops toward the end of the course of arsenical injections, he attributes to the action of the arsenic. Nicaud reports 8 cases of early, and 16 of late icterus which complicated arsenical treatment. In one case the disturbance appeared after the first injection. The date of the infection, or the dose of the drug, seemed to play no essential rôle.

The fact that these cases are so rare when one considers the large number of cases who undergo treatment, leads one to suspect that the fundamental cause lies rather in the condition of the liver at the time the drug is administered. It is conceivable that somewhat the same conditions exist as those which account for the necrosis following chloroform anesthesia. Furthermore, it seems as though many of the severer cases of jaundice follow the combined use of arsenic and mercury, a fact which accentuates this conception.

This matter of toxic reactions that appear during or after treatment—especially the intravenous—with arsenic preparations is discussed by Parnell and Fildes upon the basis of their experience with the intensive treatment with neosalvarsan (neokharsivan and novarsenobillon). Most of the cases were suffering from early syphilis and all were, except for their infections, healthy. There were 1,256 patients treated, and each received a course of six doses of 0.45 gm. given intravenously at two-day intervals. Parnell and Fildes separate the toxic reactions which they believe are due to the drug

itself from the so-called endotoxic reactions which, they say are invariable to a greater or less extent after the first and occasionally after the second injection in cases of early untreated syphilis. These reactions consist of pyrexia, which usually starts six hours after the injection, and may be accompanied by a chill or headache and nausea. The cause of this is, they say, to be found in the "syphilis toxin" which is suddenly liberated from the treponemas by the destructive action of the drug. They also exclude what they call "water fever," which is due to impure water, and which consists in a fever starting very shortly after the injection.

The symptoms upon which the authors place emphasis are pyrexia, skin lesions, headache, suffusion of the eyes, vomiting, edema, pain, herpes, rigors, delirium, coryza, nausea, jaundice, albuminuria, dryness of throat, air-hunger, salivation, cyanosis, biliuria, and a number of other minor ailments. Pyrexia, skin lesions, headache and suffused eyes both in point of incidence. The skin lesions were mostly erythematous or macular in type. Symptoms of any type occurred in but 55 of the series of cases, and for the most part they were exceedingly mild. Considering the potency of the drug and the number of patients in whom it was used, the number of complications is almost negligible.

In connection with this matter of ill effects of salvarsan it is of interest that in the Report of the Medical Research Committee, the causes are assigned to (1), toxicity of the drug; (2), errors in technic; (3), susceptibility of the patient; (4), causes unknown. Our own opinion is that given a standard preparation of the drug, and a good technic, including testing of the ampoules by immersion in alcohol before using them, the main cause is a personal susceptibility of the patient. If the drug is administered by the gravity method, then an essential part of the technic includes careful preparation of the rubber tubing.

The Committee gives hemophilia, Grave's Disease, Addison's disease, lymphatism, syphilis of the central nervous system, cardiac disease (especially myocardial), aneurysm and arteriosclerosis, severe pulmonary disease, hepatic disease, gastric and intestinal catarrh, renal disease, alcoholism, acute septic conditions and constitutional tendency to skin diseases, as calling for caution in the use of arsphenamine. As danger signals during treatment they give loss of weight, headache, insomnia, loss of appetite, appearance of renal symptoms, stomatitis, diarrhea, jaundice, and erythema. Still more information, statistically speaking, is obtained from the committee of German physicians whose report is reviewed by Meirovsky. This report deals with 13,000 injections with old salvarsan, 40,951 of sodium salvarsan, 171,826 of neosalvarsan and 64,500 of silver salvarsan. Out of the total injections of old sodium and neosalvarsan there were 12 deaths attributed to the drug (1 in 18,815); but as some of the fatalities might have been avoided by more careful precautions the chance of death is put at 1 in 56,445 (1 in 13,000 with old salvarsan, 1 in 20,000 with sodium salvarsan and 1 in 162,800 with neosalvarsan). When the dose of neosalvarsan exceeded 0.6 the deaths rose to 1 in 3000, hence the committee recommend that 0.6 be the maximum dose. Encephalitis and dermatitis were more liable to occur after an overdose.

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—P. G. W.

### *Are We in Danger of Typhus Fever?*

WITHIN the past few weeks there has been great excitement concerning cases of typhus fever which have reached New York. One would think from reading the newspapers that this is the first time this disease has within recent years found its way into our country. This is by no means true. In all probability New York City has never been entirely free from typhus fever. A few years ago it was demonstrated that Brill's disease is typhus fever. There is no evidence, so far as we know, that there have been secondary cases among citizens of this country. It is a fact that typhus fever has been permitted and is still being permitted to seep into this country both from Mexico and Europe. The following is an illustration of what has happened recently, what probably is happening now, and what quite certainly is likely to be repeated in the near future:

In the early part of July, 1920, Mrs. Doba Fischler, age sixty, and her son-in-law, Mr. Efroim Schachter, age thirty-one, left their home in a small village in Poland; after spending a few weeks in Warsaw, about two weeks in Paris and one in Havre, they boarded the Steamer La Savoe on August 21, taking steerage passage. They landed in New York on the thirtieth and disembarked at Ellis Island on the thirty-first. They say that they were sick for two days before reaching New York. At Ellis Island they were deloused, but the fact that they were ill was apparently not recognized. They reached Flint, Michigan, September 1, and at that time were quite ill. The physician called to see them made an immediate diagnosis of typhus fever and they were placed in an isolation hospital. Mrs. Fischler died on September 8. Mr. Schachter recovered and was discharged from the hospital on September 27. There can be but little doubt that these people received their infection on board the steamer.

Boyd and others showed before the War, at least before we got into the War, that typhus fever occasionally finds its way from Mexico into the very heart of the United States. Evidently, the United States Public Health Service, which usually does splendid work, has failed and is failing and may continue to fail, to keep typhus fever out of our country. If this be due to lack of authority, Congress should be asked to confer upon this Service more authority. If it be due to lack of funds, Congress should be asked for larger appropriations. With the splendid work that this Service has done in the past we are not going to believe that the seepage of typhus fever into this

country is due to lack of alertness on the part of the U. S. Public Health Service.

It seems to be quite generally believed that the introduction of typhus fever into this country is a matter of no great importance and that there is not the slightest possibility that it may gain a foothold in our land. We are not so sure about this. When we sent soldiers to Europe in 1917-1918 it was found that a large percentage of the negroes from the South and of the foreign element from the North was lousy, and even today, especially in cities where the foreign population is large, there are thousands of lousy people. Typhus fever can be kept out of the country. The Public Health Service can do this. If the Public Health Service fails, the people have a right to know why it fails.

—V. C. V.



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## ORIGINAL ARTICLES

### THE BACTERIOLOGY OF CHRONIC NONTUBERCULOUS LUNG DISEASE

BY HORACE GREELEY, M.D., AND MAE BRERETON, BROOKLYN, N. Y.

SOMETIME ago, through the kindness of the medical superintendents of the Sea View Hospital and the Otisville Sanatorium (Dr. E. S. McSweeney and Dr. W. L. Rathbun) specimens of sputum were obtained from all the persistently negative, as regards the presence of tubercle bacilli, cases then in these institutions. To these were added a few cases under the care of various physicians in the city, and we then proceeded in an attempt to work out their bacteriology. It is thought that the findings are of sufficient interest to justify the present article.

A short summary of the histories and lung conditions of the persons from whom the specimens came is given in a table, presented herewith.

#### CULTURE MEDIUM USED

Knowing that an attempt to isolate, on ordinary media, all the morphologically different forms from so many specimens would in all probability, swamp one with work, it was determined to try what could be done with selective media. As, in examining smears of sputum from such cases, yeast bodies and mycelium-like fragments had often been observed, it was resolved to try media of acid reaction, upon which it was known such forms could be grown.

In order to obtain a standard medium which would act selectively, a series of twelve tubes, each containing 2 c.c. of neutral nutrient agar was prepared, and to each of these, while liquid, sterile 10 per cent lactic acid solution was added—lactic acid, if heated in solution with agar, destroys its solidifying power. The lactic acid was added in increasing strengths to the successive tubes of the series, and the set, slanted, was then inoculated with the sputum of a case (N.S.) showing many yeast-like bodies, and incubated for 48 hours. At the end of this time it was found that tube 6, containing approximately 0.3 per cent of lactic acid, represented the minimum strength of the latter which

could safely be relied upon to prevent growth of organisms requiring neutral or alkaline media, and which would also give a good growth of yeasts.

In looking over the literature, preparatory to writing the present report, we ran across an article by MacDonald<sup>1</sup> which calls attention to a recommendation of Duggar's<sup>2</sup> that lactic acid be employed to isolate pure strains of fungi. Duggar states that 0.5 per cent of lactic acid is sufficient to prevent the growth of contaminating bacteria. MacDonald found that higher fungi, including actinomyces, sporothrices, and blastomyces, were more than twice as resistant as the ordinary known bacteria, while *Mucor mucedo* and *Aspergillus flavus* were decidedly tolerant, growing in media containing several per cent of lactic acid.

It was also recently learned that manufacturers of commercial yeast first inoculate their medium with lactic acid bacilli which, after a period of incubation, are killed off by heat before the yeast is planted. Such a proceeding has an effect similar to that produced by an addition of the acid, and is undertaken to prevent organisms, other than the yeast, from growing in the medium.

Upon this 0.3 per cent lactic acid nutrient agar we planted smears of sputum from all the cases studied, and incubated tubes from each case at both body and summer temperatures (70 to 80° F.) To simplify the presentation of the results obtained we shall give them in groups, corresponding to the organisms isolated.

#### PENICILLIUM GLAUCUM GROUP

The *Penicillium glaucum* is a most interesting organism. Besson<sup>3</sup> says that it is one of the commonest of moulds, and mentions that it is pathogenic for dogs, rabbits, and lambs. He also quotes reports that it had been found in several cases of middle ear inflammation.

Roequefort cheese owes its peculiar qualities to this organism, to the spores of which the green lines in the cheese are due.

Canio<sup>4</sup> reported the case of a man of thirty-one, who suffered a severe attack of what simulated pulmonary tuberculosis. Examination of the sputum disclosed no tubercle bacilli, but numerous elements which proved to be the *Penicillium glaucum*. He states that biologic tests and serum reactions confirmed the supposed causal relationship of the organism isolated, and that the mycosis was reproduced by injecting rabbits with the sputum of the case.

There are some other reports in the literature of infectious lung disease probably due to this organism, so far as one can judge from the partial descriptions given of the fungi isolated; and even one or two of the illustrations accompanying these reports are strongly suggestive of the *Penicillium glaucum*. However, as other names were applied to the organisms, we shall not quote the cases in this connection.

Fourteen cases (Numbers, 2, 4, 8, 11, 12, 14, 15, 21, 22, 23, 24, 25, 26, 30) of those included in this report gave cultures proved to be of the *Penicillium glaucum*.

#### SPUTUM APPEARANCES

Examination of smears of the sputum of these fourteen cases showed many large and small coccoid bodies, some having the appearance of being encapsu-

lated; in nine instances, also many typical yeasts; and, in many, a few of what might be called large granular strepto-bacilli, or segmented mycelium, from which small coccoid bodies, joined in pairs, could frequently be found issuing. With the typical yeast forms, large, cone-shaped aggregations of what seemed to be branching yeasts were observed in several instances. Forms intermediate in appearance between yeasts and cocci were common. Some of the yeast bodies were oval and some spherical, and some showed the various internal markings frequent in these bodies. The sputum of case 8 (included as a control), besides the forms described also contained tubercle bacilli. In this connection we call attention to observations of Sanfelice<sup>3</sup> to the effect that the lungs of tuberculous cattle constantly contain blastomycetes and other fungi. Among the former he found species pathogenic to laboratory animals. Furthermore, he states, that animals inoculated with these blastomyces, in addition to tubercle bacilli, died sooner than those inoculated with tubercle bacilli only; and that the lesions showed both organisms.

In several of these fourteen cases (for instance, Nos. 12 and 22) the original jar of sputum was left standing, at room temperature, for a few days, and another smear of the sediment then examined. At this time the organisms, originally of the size of ordinary micrococci, were found to have enlarged to distinctly yeast size and appearance, without having lost any of their dye-taking qualities. Seven out of twelve of the jars containing the specimens of this group that were left standing, as above mentioned, developed, after about two weeks, a thick surface growth of the sporulating fungus. On two specimens the surface growth did not sporulate, and microscopically suggested large streptococci. On two others a creamy growth appeared, which proved to consist of yeast-like branchings. On one specimen no growth, whatever developed.

#### CULTURES

Plantings, from the sputa of the fourteen cases, on the lactic acid agar, incubated at 70° to 80° F., gave after forty-eight hours, opaque white colonies, which, upon microscopic examination, showed a pure culture of forms varying from typical yeasts to beginning hypha formations. After 72 hours, sporulation became apparent, and, within the next twenty-four hours, the entire growth became dark green. Microscopic examination showed the *Penicillium glaucum*.

Cultures upon the same media, planted at the same time as the above, but incubated at body temperature, gave yeast forms, which, although some showed hypha-like branchings, did not develop to the conidia-producing fungus. Transplants of the cultures showing marked tendency to mycelium production to solidified Loeffler's serum, incubated at the same temperature, grew in the bacillus form. Transplants of the yeast forms to lactic acid agar or to acid sugar agar, incubated at 70° to 80° F., gave, in most instances, the conidia-producing fungus. It was noted that in several instances in which a yeast-form culture of this organism had been carried through several generations without mycelium production the form apparently became fixed so that we could not cause subsequent transplants to produce conidia. All the forms that became

aborted in this way showed appearances within the yeast-bodies and the occasional hypha produced similar to what have been denominated ascospores or endospores.

Cultures of the *Penicillium glaucum* (grown on either the lactic acid agar, or on sugar agar, such as 2 per cent maltose agar) which had been incubated at 70° to 80° F. till the surface showed complete sporulation, as evidenced by the dark green conidia masses, were taken, and transplants made as follows:

In 2 per cent maltose bouillon and incubated at 70° to 80° F. for 24 hours. From the second generation so grown transplants were made to Loeffler's solidified serum, and incubated at body temperature for 24 hours. Plants on the latter medium, made from the conidia masses of the mother cultures were also made and incubated at the same temperature (98° F.).

The bouillon cultures, at the end of 24 hours were diffusely clouded, and, microscopically, they showed cocco-bacillus forms only. The transplants to Loeffler showed a bright lemon-colored growth, of creamy consistency, of a small bacillus form. While this latter could be propagated indefinitely in the same form, we were unable to cause it to develop to the sporulating fungus. Plantings of conidia upon Loeffler did not grow at body temperature.

Cultures of the *Penicillium glaucum*, after propagation as the sporulating form (at 70° to 80° F.) for some time, gradually lost their ability to readily grow in the small bacillus form, and it was noticed that the hyphæ of cultures refractory in this way usually failed to show the central granules which, in other cultures, so frequently may be seen escaping from a broken hypha.

Thus it seems demonstrated that a higher fungus may be grown in the form of a morphologically perfect bacillus, by definite procedures, and that this bacillus is so permanent that it might easily be classed, by one unfamiliar with its antecedents, with bacilli which our present knowledge leads us to believe are as definite varieties of the earthly flora as any of the plants which claim the interest of the horticulturist. As previously recounted, this same fungus has a yeast form, which, however, is a morphological variation common among the higher fungi, as many authorities testify.

The above described variations in the *Penicillium glaucum* are excellent illustrations of persistence of characteristics acquired in a single bacteriologic generation, which probably represents ten or a dozen in actual descent, assuming that each organism divides at the age of two hours and that the culture is incubated 20 to 24 hours. Although the principles of evolution demand that such changes should occur, and most students of biology and natural selection believe them to be the key to the varied flora and fauna of nature, yet many bacteriologists will hesitate to accept the above statements.

#### CLINICAL CHARACTER OF CASES

(See the accompanying table)

One of the cases showing this organism, also had tubercle bacilli in the sputum (Case 8). The illness was said to have been of less than a year's duration, but the physical signs of lung disease were the most extensive of all in this group. Two cases (1 and 11) were complicated with syphilis.

Tuberculin tests were performed in but two of the cases (2 and 12) and were reported as giving local-positive reactions.

Excluding the case showing tubercle bacilli and the two syphilitic cases, and considering the eleven remaining, we are struck by the long duration of the malady and the slight amount of lung damage done. For instance, Cases 21, 22, and 14 had had a duration of 16, 12, and 9 years, respectively, while the corresponding lesions were described as "infiltration right apex" (Case 21), "slight fibrosis upper lobe" (Case 22), "infiltration upper lobes" (Case 14). Cases described as "chronic bronchitis" and as "bronchial asthma" (Numbers 24, 25, 26) were in persons aged, respectively, seventeen, thirty-six, and fifty, but had had a duration of from two to five years. Case 30 was of acute bronchitis, which had lasted ten days.

Case 23 was that of a little Syrian boy, of nine years, who died of his infection several months after we first examined his sputum. This latter was very watery and contained whitish flakes, a description which applies to most of the sputa of the cases reported in this paper. Smears made of this boy's sputum, on every occasion upon which it was examined, during the course of his illness, showed numerous spherical yeast bodies, in ones and twos; many coccoid forms; and others intermediate between these and yeasts; besides a few of the large streptobacillus-like growths which, we have stated, were probably mycelium fragments. Other bacterial forms present were pneumococci, and bacilli resembling Friedlander's.

This case, which the attending physician, on request, kindly brought for examination, showed evidence of but slight fibrosis of the upper lobes of the lungs, and some swelling of the glands of the neck. He coughed a good deal, showed but slight febrile reaction, little loss of weight, but a considerable amount of debility. Later, the attending physician reported that the boy grew progressively weaker, his neck glands slightly larger, and that he died from what is sometimes denominated asthenia, some two months later. No autopsy was obtainable.

#### EFFECTS OF VACCINES

In three of the cases, in Sea View Hospital, showing the *Penicillium glaucum* in the sputum, Dr. E. S. McSweeney very kindly tried autogenous vaccines, that we prepared, containing about 3000 million of the yeast form of the fungus in each cubic centimeter. In one case (R.G.) three injections, 8 days apart, were given, of, respectively, 0.055, 0.10, 0.15 c.c. Each time a local reaction was elicited. Injections were stopped, in this case, as the patient's general condition was improving under the mercurial treatment given for syphilis, from which, as recorded, the patient was suffering. In another case (H.S.) that showing tubercle bacilli in the sputum—two injections of the vaccine were given, both followed by local reactions, and the second (of 0.10 c.c.) by a severe hemorrhage. The other case (M.C.) had eight doses, running from 0.055 to 0.4 c.c. over a period of two months. As the injections gave rise to marked local and general reactions (including rise of temperature and increased cough and expectoration) the treatment was stopped. It should be noted that a marked reaction also took place in the enlarged axillary gland

SPUTUM NEGATIVE (FOR TUBERCLE BACILLI) CASES—TABLE GIVING LUNG CONDITION, ETC.

NO.	NAME	SEX	AGE	OCCUPATION	DURATION OF DISEASE, YEARS	LUNG CONDITION, AND COMPLICATIONS	SPUTUM EXAMINATIONS FOR TUBERCLE BACILLI		TUBERCULIN TESTS
							NO. OF EX- AMINATIONS	RESULT	
1	C.B.E.	M.	24	Engraver	1½	Infiltration right apex, slight fibrosis left apex. Lesion moist.	Numerous, negative		11/19/16 von Pirquet (O. T. 100% and 50%). Area of inoculation reddened; defi- nite induration. No focal or con- stitutional reaction.
2	M. C.	F.	22	Dressmaking	1½	Probable infiltration apices and left upper lobe. Lesion moist.	36 negative		9/28/16 O. T. O. 1 mg. intra-cuta- neously; marked local reaction only.
3	J.D.	P.	18	Student	2½	Infiltration apices and right upper lobe. "Tuberculous" laryngitis. Moist.	2 positive		None
4	R.G.	P.	40	Housework	3	Fibrosis right apex; infiltration left base (not typical)—fibrosed? Lesion moist. Wassermann test: (++++)	2 negative		None
5	C.B.	F.	42	"	1½	Fibrosis right apex; infiltration left apex and upper lobe. Lesion moist. "Tubercular" laryngitis.	2 "		None
6	N.H.	P.	54	"	1	Infiltration apices and upper lobes "Diffuse asthma." Lesion moist.	2 "		10/15/16 O. T. O. 1 mg. intra-cuta- neously; sharp local reaction also, some general reaction next day.
7	F.Q.	P.	64	"	8	Infiltration left apex and upper lobe; fibrosis right apex. "Diffuse asth- ma." Lesion moist.	2 "		8/6/16 von Pirquet (O. T.) posi- tive—sluggish reaction.
8	H.S.	F.	20	"	1½	Signs of disease obscured by emphy- sema. Right lung indurated. Lesion moist.	2 positive		None.
9	B.S.	M.	40	Clerk	3	Infiltration of apices.	1 positive		9/21/17, von Pirquet (O. T. 50%) 24 hour 5x3.
10	J.B.	M.	16	School boy	2	Infiltration left upper and right apex	2 negative		11/19/16, von Pirquet (O. T. 100%) slightly positive.
11	P.D.	M.	40	Carpenter	2	Infiltration of both apices. Syphilis.	Numerous, always nega- tive		None.

SCUTUM-NEGATIVE (FOR TUBERCLE BACILLA) CASES—TABLE GIVING LUNG CONDITION, ETC. CONT'D

NO.	NAME	SEX	AGE	OCCUPATION	DURATION ILLNESS YEARS	LUNG CONDITION, AND COMPLICATIONS	SCUTUM EXAMINATIONS FOR TUBERCLE BACILLA		TUBERCULIN TESTS
							NO. OF EX- AMINATIONS	RESULT	
12	E.S.	M.	30	Clerk	2	Infiltration of right apex and pleurisy of right base.	5	negative	11/19/16, von Pirquet (O. T. 100% and 50%) 21 hours, positive.
13	G.R.	F.	39	Housework	2	Infiltration right upper lobe, moderate infiltration left upper.	Numerous	negative	None.
14	F.H.	F.	32	Nurse	9	Infiltration upper lobes.	42	negative	None.
15	C.S.	F.	24	Housework	4	Infiltration of right apex, extending into lobe.	16	negative	None.
16	H.W.	F.	22	Clerk	2	Infiltration of left apex.	14	negative	None.
17	W.M.F.	F.	38	Operative	2½	Infiltration of apices, extending into right upper lobe.	16	negative	None.
18	A.M.C.	M.	30	Laborer	1	Infiltration of right apex; pleurisy right base.	10	negative	None.
19	I.K.	M.	38	Attendant	2	Old dry cavity right apex; infiltration right lower lobe, "Spots," left apex and lower lobe.	41	negative	None.
20	A.T.	M.	30	Orderly	½	Infiltration of right apex extending into upper lobe.	22	negative	None.
21	C.G.	M.	44	Clerk	16	Infiltration of right apex.	17	negative	None.
22	M.L.	F.	61	Housework	12	"Diffuse rates" (slight fibrosis upper lobes?).	Numerous	negative	None.
23	N.S.	M.	9	School boy	¾	"Diffuse rates" (slight fibrosis upper lobes?). Slight glandular involvement, neck.	4	negative	None.
24	M.S.	M.	17	Student	2	Chronic bronchitis.	8	negative	None.
25	M.O.	F.	36	Housework	5	"Bronchial asthma."	4	negative	None.
26	A.S.	M.	50	Agent	4	Chronic bronchitis.	6	negative	None.
27	J.C.H.	M.	60	Writer	16	"Bronchial asthma."	12	negative	von Pirquet, negative.
28	M.W.	M.	24	Clerk	3	Infiltration upper lobes.	Numerous, negative (?)	negative	None (?)
29	A.B.	F.	25	Stenographer	2	Chronic bronchitis.	Numerous, negative (?)	negative	None.
30	M.S.	F.	24	Dancer	10 days	Acute bronchitis.	2	negative	None.
31	M.W.	M.	35	Clerk	3 weeks	Acute bronchitis.	2	negative	None.

of this patient. The chief interest of the experiment with vaccines was, of course, the reactions elicited, which may be regarded as confirming the sputum and culture findings.

An apparently successful use of an autogenous vaccine in the treatment of one of the cases of this group was reported by the attending physician. Here a similar preparation was used and the patient, a houseworker, sixty-one years of age, who had suffered from chronic bronchitis for twelve years, was entirely rid of her symptoms (see Case 22—M.L., in table).

#### PENICILLIUM GLAUCUM WITH MODIFIED SPORANGIUM

In Case 31, examination of the sputum showed many organisms in the form of diplococci and diplobacilli.

Lactic acid selective media, at 98° F., within 48 hours, gave a growth of yeasts. By the third day, cultures grown at 70° to 80° F. produced a brown sporulation. Microscopic examination showed a mycelium, with septate aerial hyphae, bearing sporangia which appeared to be divided into 4 to 12 compartments. This organism grew readily on the other media, including Raulin's mentioned above, and under similar conditions. As with the preceding fungi, entirely bacillary forms could be developed. After about a year's cultivation, this organism lost the form of sporangium described and produced the free rows of conidia typical of the *Penicillium glaucum*.

#### CLINICAL CHARACTER OF THE CASE

This case presented clinically, according to the attending physician, all the features of a severe acute bronchitis, in which the lung symptoms were so marked as to lead him to have the sputum examined in order to rule out tuberculosis. The case entirely recovered, within a few weeks, under the usual treatment.

#### PATHOGENIC MUCORS

*Mucor Corymbifer*.—Besson (previously quoted, page 677) says: "It is pathogenic for rabbits. It has been found in man in the ear and in the pharynx (Siebenham, Huckel, and others). One case of generalized mucor mycosis in man, in which the symptoms were of a typhoid nature, was attributed to this parasite (Paltanuf); and it would seem that the two cases of human mycosis (pulmonary mucor mycosis) described by Furbinger, and referred to above, should be attributed to this species rather than to *mucor mucedo*."

In cases 9 and 27 of the present series, the sputum, upon examination, showed a great many large coccus forms, a few yeast bodies in couples, and bacillus-like pieces of mycelium. On standing, at room temperature, for some days, a surface growth developed on the sputum, showing coal black sporulation. This proved to be the same fungus described in the following, isolated by means of the selective media.

On the lactic acid medium there developed, in the first generation, in forty-eight hours at 70° to 80° F. (later, in replants, in 24 hours) a black sporulation, which, microscopically, had the appearance of that shown by the *mucor corymbifer*. On the same medium, at body temperature, distinct yeasts



were produced, which, however, were mixed with branchings of the mycelium, as this organism developed to sporulation at both temperatures, and on all medias tried, which included Loeffler, plain nutrient agar, and Raulin's. Beady bacillus forms were developed by transplants and retransplants on Loeffler (incubated at body temperature) made before the sporulation stage had been reached.

#### CLINICAL CHARACTER OF CASES

Case 27 was a man of 60 years of age, weighing 186 pounds, a writer by profession. He had been treated for what his physician diagnosed as "bronchial asthma" for sixteen years, and, recently, had lost some weight and had developed a most persistent and harassing cough. Besides the usual medications, his physician used a vaccine, which we made of the organism isolated, but without result. He informed us that, although he had pushed the dosage, no reaction, whatever, could be obtained. Patient was sent to a sanatorium and, when heard from last had made slight improvement.

In the other case showing the same organism (No. 9, B.S., Sea View Hospital) Dr. McSweeney gave ten injections of a vaccine (autogenous in yeast form) over a period of two months, without other result than regularly recurring local reactions.

#### ASPERGILLUS FUMIGATUS

Besson (previously quoted, page 695) says of this fungus: "Laulanie has shown that *Aspergillus fumigatus* is capable of producing a condition of pseudotuberculosis when inoculated, experimentally, into animals. Cases of pseudotuberculosis, due to an aspergillus, have also been recorded by several observers as occurring in the human subject. *Aspergillus fumigatus* has been found in infections of the ear and nasopharynx, and in cases of keratitis with hypopyon following wounds of the eye caused by vegetable tissues.

"Pigeon-crammers are very subject to aspergillary pseudotuberculosis. In pigeons there is often a 'chancre' on the buccal mucous membrane due to an aspergillus. The disease is also found in hair-combers who use flour of rye—which is often infected with spores of aspergillus—for removing the grease from hair. In human aspergillary pseudotuberculosis the fungus is often associated with the tubercle bacillus. *Aspergillus fumigatus* has also been found in the lesions of pneumomycosis in the horse and cow. Renon has found that by sowing millet seeds, vetch, oats, maize, wheat, and other varieties of corn on appropriate media, cultures of various species of aspergilli can be obtained, the commonest being *aspergillus fumigatus*."

The sputum of Case 28 showed many diplococci and large capsulated streptobacilli—the latter might be called fragments of mycelium.

Upon the lactic acid selective media, yeast forms developed at body temperature; and the same went on to branchings and mycelium production, within 72 hours, at 70° to 80° F. Good growth was obtained on Raulin's medium. Bacillary forms were produced on Loeffler, inoculated at body temperature, after a few transplants.

## CLINICAL CHARACTER OF CASE

Clinically this case was reported to have all the characteristics of a chronic "lunger", and to have spent some time at Saranac Lake, previously, with more or less benefit.

## ABORTED FUNGI

*Sputum.*—Examination of smears of the sputum of the remaining thirteen cases, of the series of thirty-one, gave results as follows:

Cases 1, 7, 18, 20, coccoid forms, only.

Cases 13, 29, coccoid and bacilloid forms.

Cases 3, 5, 6, 10, 16, 17, 19, coccoid forms and yeasts.

*Cultures.*—Upon the lactic acid selective media, under the conditions described in the foregoing, plants made from the sputa of eleven of these thirteen cases developed yeast bodies. Cultures from Cases 1 and 3 (on this media) did not grow, although many yeasts were present in the sputum of Case 3. Upon alkaline or neutral nutrient agar, cultures from the two last mentioned cases showed beady bacilli and many oval, non-stain-taking forms.

All of the yeasts (in the eleven cultures showing these bodies) showed a tendency to the production of mycelium, as many ground-hyphae were produced, but none developed aerial hyphae or terminal spores. Upon Loeffler, at 98° F., all the yeast cultures, except those from Cases 5 and 10, readily developed a bacillus form—this, evidently, being simply the continued propagation of the hyphae, with slight modifications caused by the conditions, and in the course of which the filaments became more slender and tended to break up at the septa.

Cultures from cases 5 and 10, both showing many yeast forms in the sputum, held very persistently to this form, showing only an occasional hypha, during more than a year of cultivation. A mycelium-producing, or bacillus, form was finally developed by repeated transplants on 2 per cent maltose agar, incubated at 70° to 80° F., until a feathery edge was observed on the outer edge of the colony masses. This feathery edge was found to consist entirely of mycelium. Careful transplants were made, so as to avoid carrying over any yeast forms, to Loeffler, and incubated at body temperature, with the result desired—cultures showing only filaments, which tended more and more to break up into the bacillus form, finally becoming typical bacilli.

No culture from the cases included under this heading developed into a definite higher fungus, although some showed long streptothrix-like branchings which, in two instances, showed what might be called terminal buds or spore-pods.

In Case 10 (Sea View Hospital) Dr. McSweeney tried an autogenous vaccine, in the yeast form, giving twelve small doses over a period of three months. No result, beyond a regularly recurring local reaction, followed.

## CONCLUSIONS

1. Various higher fungi may be cultivated from the sputa of chronic nontuberculous, and probably from many cases of tuberculous lung disease. This is most easily done through the use of selective media.

2. Evidence is adduced that such organisms are extremely numerous in such sputas, and that they probably are potent in the disease process, acting alone in some cases, and in cooperation with the tubercle bacillus in a few others.

3. As of assistance in the eventual complete identification and classification of the organisms to be found in sputa, it is thought that important evidence has been adduced of the extreme pleomorphism of certain fungi, probably common therein under forms morphologically representing yeasts, cocci, and bacilli.

#### REFERENCES

- <sup>1</sup>Jour. Infect. Dis., July, 1917, p. 322.
- <sup>2</sup>Fungous Diseases of Plants, 1909.
- <sup>3</sup>Pract. Bact. Microbial. and Serum Therapy, English ed., 1913, p. 700.
- <sup>4</sup>Jour. de Med. de Bordeaux, June, 1918.
- <sup>5</sup>An. d'Igiene, (Rome) 1918, xxviii, 41.

## ON THE PREPARATION OF METAL SALTS OF THIOGLYCOLLIC ACID\*

BY C. N. MYERS, PH.D., WASHINGTON, D. C.

A DESIRE to study the relative toxicity of heavy metals, when combined with a common ion, and the effect of these compounds upon parasites, suggested the preparation of an extensive series of heavy metal salts suitable for a biological investigation under uniform conditions.

The widespread occurrence of diseases caused by parasites, in animals and in man, has led to numerous efforts to improve the treatment of these diseases through the preparation of chemical compounds which can be administered safely and effectively. In order to meet the demands of this parasitic invasion, a definite knowledge of the chemotherapeutic agents at our disposal must be obtained. A knowledge of the difficulty of curing cases, in the incipient as well as in the later stages of development, makes it necessary to produce chemotherapeutic agents which are potentially powerful enough to destroy the parasites initially, and later in the reproductive cycle. Furthermore, the drug must show marked efficiency in sterilizing the tissues which have undergone this parasitic invasion.

In order to replace any of the substances now employed for this purpose, a drug must show a marked superiority in adaptability for administration, either intravenously or subcutaneously. The solubility of the drug in the presence of the body fluids, and the tendency toward decomposition are two other chemical factors necessary to observe in the preparation of the compound to be used for this purpose. The fact that the free acid salts of thioglycollic acid are quite insoluble in water renders it impracticable to use this combination. To obviate this difficulty the sodium salt was used in the study upon animals. The study of arsenicals and antimonials, and various dyestuffs, has been of the greatest importance; the work of Ehrlich, his co-

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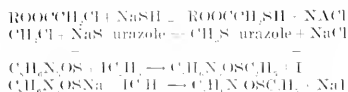
workers and contemporaries, is so well known that it needs only passing reference in respect to this study of heavy metals. In this paper will be found the methods of preparation used by the author in making the heavy metal salts of thioglycollic acid. The main desire was to obtain compounds of simple combination and of a high degree of purity. Controversies over constitution and complex formations are only of passing interest in this connection. The following characteristics determined the choice of this acid: first, it is a comparatively strong acid; secondly, it very easily forms salts with heavy metals; thirdly, the anion is practically inactive physiologically, thus giving a total effect due entirely to the heavy metal; lastly, it gives a sodium salt readily soluble in water.

#### HISTORICAL

Carius (1862) investigated the action of the alkali sulphides upon monochloroacetic acid, and in this investigation succeeded in producing thioglycollic acid. He believed that concentrated solutions of the two compounds were necessary, but one must remember that the effects of mass law and dilution were scarcely thought of at this time. Likewise the theory of ionic and molecular activity was not yet conceived. Carius recommended the use of three parts of monochloroacetic acid and five parts of a very concentrated solution of potassium sulphhydrate. The mixture was heated to boiling, on an oil bath, and allowed to remain at this temperature 3 to 4 hours. The acid was precipitated as the barium salt, and later obtained by decomposing this salt with hydrochloric acid.

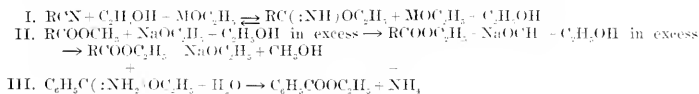
Claesson (1877) described the method generally in use at present for preparing this acid. He first pointed out the fact that salts of three different types could be formed and, furthermore, showed that complexes of these types could likewise exist. The constitution of these complexes has been the source of considerable controversy. Claesson here comprehended the relation of mass law to the yield, but did not realize it until his later work with Carlson, which showed definitely how hydrolysis, mass law, and dilution worked together to give almost quantitative results. Claesson and Carlson (1906) found that it required only 8 to 10 minutes to reach equilibrium. The fact which is important is so to regulate the masses of salts and their solvents that the un-ionized molecules can react with the greatest efficiency.

In this connection we have an excellent analogy in the quantitative studies of Aeric (1906) and his collaborators in which they studied the reactions of ions and molecules at constant temperature with sulphur containing compounds. Reactions of the following nature show these facts:



In the above case it was shown that the value for  $K_i = 0.455$ , and  $K_m = 0.170$ . In other words the ionized sodium 1 phenyl-3-thiouazole reacts 2.5 times as rapidly as the undissociated part. These facts are obtained from the well-known equations for reaction velocities used in the Organic Laboratory

of Johns Hopkins University. These same facts hold true in the case of nitriles catalyzed by sodium, potassium, and lithium ethylates in alcoholic solutions as:

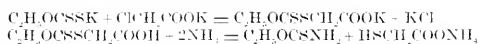


We are beginning to realize the importance of the true mechanism of chemical reactions and are preparing our compounds accordingly. The transition of thioglycollic acid into thiodiglycollic acid is a most striking illustration of this point.

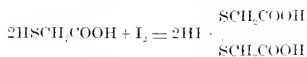
Liebermann and Lange (1881) in studying the constitution of the phenylsulphydantoins, prepared the lead salt of thioglycollic acid, but their analyses did not prove the existence of a pure lead salt of the composition  $(\text{C}_2\text{H}_3\text{SO}_2)_2\text{Pb}$ .

Ramberg (1906) studied the constitution of the antimony salt, which seems to be the best worked out of any of the thioglycolates. There is an almost uniform agreement that it is a lactone form. The work in this laboratory confirms this point of view.

Bilmmann (1905), in studying the xanthogenic acids, found a method of saponifying them with ammonia, with the formation of thioglycollic acid. The method employed by Bilmmann consisted essentially in carrying out the reaction according to the following equations:



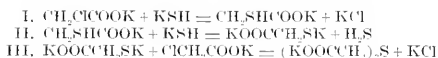
Eighty grams of potassium xanthogenate (one-half molecule) were dissolved in one hundred grams of water, and twenty-eight grams of sodium carbonate were added. A neutral solution of monochloroacetic acid was prepared by dissolving forty-seven and a half grams of the acid in 200 grams of cold water and neutralizing with potassium hydroxide. The two solutions were put together and allowed to stand overnight at ordinary temperature. The mixture was acidified with one hundred cubic centimeters of thirty per cent hydrochloric acid. Xanthogeniacetic acid separated out as a heavy yellow oil, the yield being 93 per cent. Forty-five grams of the xanthogeniacetic acid are dissolved in a mixture of 40 c.c. of concentrated ammonia water and 200 c.c. of absolute alcohol. The mixture is allowed to stand in a stoppered flask, at room temperature, for 48 hours. It is evaporated with alcohol and ammonia on a water-bath. The mixture is then acidified with hydrochloric acid gas and the thioglycollic acid extracted with ether and purified in the usual manner. There is a yield of 70 per cent in this case. The acid is determined, quantitatively, by determining the amount of iodine used in the condensation of thioglycollic acid into thiodiglycollic acid:



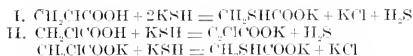
Bilhamm also prepared other thioacids, both aromatic and aliphatic, by using this same principle.

The cause of the formation of thioglycollic acid is found in the weakly acid character of the SH group in thioglycollic acid, which group is partially converted into SK by alkaline solution of potassium sulphhydrate.

For example:



In the production of a quantitative yield, the reaction should proceed as follows:



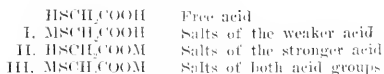
In either case the yield is good, if the reacting substances are kept within these proportions. The acid may first be neutralized, and then treated with 1 molecule KSH or with 2 molecules KSH as in equation I.

Claesson and Carlson (1906) studied the replacement of Cl by SH in the case of monochloroacetic acid and found the following interesting values, which in turn show the direct application of mass law and dilutions:

$\text{ClCH}_2\text{COOH}$ in parts of water	KSH	$\text{HSCH}_2\text{COOH}$ grams formed	Yield in per cent
0	50 per cent	2.81 g.	57.7
1	50 per cent	3.13	64.2
2	50 per cent	3.60	73.9
3	30 per cent	4.45	91.3
5	20 per cent	4.71	96.7
6	10 per cent	4.85	99.6

From this table the increase in yield is very marked in the dilute solutions. In work carried out in connection with this investigation, a yield of 90 per cent was the best that was obtained. This table is of importance in that it shows very clearly how we may secure the best yield of acid.

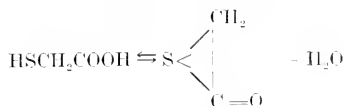
By examination of the formula for thioglycollic acid it will readily be seen that we are dealing with a dibasic acid containing a weakly acid group, RSH, and a stronger carboxylic acid, RCOOH.



Thus it is easily seen that there may be a large series of compounds formed depending largely upon the method of preparation. In this investigation the preparations from the heavy metals have been essentially that of Type I, while the alkali metal replaced the hydrogen ion of the carboxylic group.

Properties of thioglycollic acid (mercapto-acetic acid, monosulphoacetic acid). It is a viscous fluid with a specific gravity at 20° C. of 1.3253. It is purified by vacuum distillation, and the following data are given by Bilhamm for distillation: 123° C. 29 mm. pressure; 107-8° C. at 16 mm. pressure; 115°

C. at first, then 103.5° C. at 14 mm. pressure. In 100 per cent thioglycollic acid the following transformation takes place very easily.



Heat easily transforms it into thiodiglycollic acid, which is a solid of unpleasant odor. Thioglycollic acid has an odor very similar to that characteristic of the skunk. It is soluble in water, alcohol and ether. A description of the other properties and the salts is found in Beilstein (1893).

Ostwald, in his work on conductivity, found the value of  $K$  to be 0.0225 for thioglycollic acid. Claesson and Carlson (1906) give the following values for conductivity data:

V	$\eta$	$\infty$	K
10	20.27	0.0527	0.0293
50	43.70	0.1138	0.0288
100	60.29	0.1576	0.0292
500	120.84	0.3147	0.0289
1000	158.78	0.4135	0.0291
2500	217.27	0.5658	0.0295
K = 0.0291			
$\mu \infty = 384$			

This table shows that we are dealing with an acid that, in N 2500 solution, is only about 56 per cent dissociated. Since this is the case, about 44 per cent remains molecular, and it is of interest, physically, to observe that we have here a reaction in which molecules and ions both take part, in order to give us a quantitative yield.

Various methods of preparing the metallic compounds have been suggested. In brief, it can be stated that the chloride or the oxide is generally used. However, a deviation from this procedure has been necessary, and advisable, in preparing salts of the rare elements, in many cases where hydrolysis or decomposition takes place readily. The mixed salts obtained by other investigators, as also any other salts, will be discussed briefly in this paper.

It was found advisable to make the free acid in small lots. One hundred gram portions of monochloroacetic acid were dissolved in 500 grams of distilled water, and this was treated with the theoretical amount of potassium sulphhydrate, where two molecules of sulphhydrate are used with one of monochloroacetic acid. A large flask was used for mixing, and this was kept under a good hood. After ten minutes, with frequent shaking, the contents were placed on a steam bath until the excess of hydrogen sulphide was volatilized. The solution was concentrated *in vacuo*. Barium chloride was added in excess, and the solution allowed to cool. Success in securing the barium salt was poor. However, by treating with HCl and extracting with ether, good yields of the free acid were obtained. The acid was placed over calcium chloride and then purified twice by vacuum distillation. Extraction is continued until no further test for thioglycollic acid is obtained. The test employed, consists in the use of a few cubic centimeters of the solution in question, diluted to 15 or 20 c.c. Add two drops of ferric chloride and then a drop

of ammonia; a deep, Burgundy red will indicate thioglycollic acid. Dithioglycollic acid does not give this reaction. The pure product is kept in vacuum desiccators until used. The following metals were used in this investigation: antimony, arsenic, bismuth, cadmium, cerium, chromium, cobalt, copper, gold, iron, lead, manganese, mercury, molybdenum, nickel, platinum, rubidium, silver, tellurium, thallium, tin, titanium, tungsten, uranium, vanadium and zinc. These elements were obtained as pure as possible either in the form of the metal or of its soluble salts.

#### BISMUTH—SODIUM THIOLYCOLLATE

Ten grams of fresh thioglycollic acid were exactly neutralized with sodium hydroxide, using phenolphthalein as an indicator. It is always necessary to dilute the free acid with water and then slowly neutralize with the alkali. Unless these steps are taken, the heat liberated by the reaction will convert the acid into thiodiglycollic acid. A slight pink color appears near the neutral point. The bismuth salt was prepared by treating bismuth suboxide with the sodium salt of thioglycollic acid. The suboxide was prepared by the method of Schneider (1850). The suboxide was suspended in an alkaline solution and the solution of sodium thioglycollic acid added to it in the cold, for the reason that the bismuth salt would have a tendency toward decomposition or oxidation of the suboxide. No perceptible reaction took place and, after a couple of hours, the mixture was heated to  $90^{\circ}\text{C}$ . on a water-bath with considerable shaking. The flask was removed and the shaking was continued for one hour. A slight excess of sodium thioglycollate was added, and the excess of  $\text{Bi}_2\text{O}_3$  filtered off. A clear canary yellow solution remained. By cooling a sample of this solution in a freezing mixture, the crystalline sodium salt appeared. The free acid salt of bismuth was obtained by decomposition with hydrochloric acid. Considerable care should be used in this process, for the reason that the free acid salt is soluble in an excess of free mineral acid. The canary yellow precipitate is filtered off and dried in a vacuum desiccator, after washing it free of hydrochloric acid. The dry acid salt retains its canary yellow appearance even after long standing. The solution is easily changed by the action of light, with the formation of a dark precipitate which proved to be bismuth sulphide; exposure to air produced the same result. These two facts prove that all solutions for therapeutic purposes must be made up just before use.

A second experiment was carried out by using the oxide usually found in commerce,  $\text{Bi}_2\text{O}_3$ . The procedure was the same as outlined above and the product was the same. A sample of each product was placed in a melting point tube, and it was found that softening took place at  $107^{\circ}\text{C}$ . in each case. At  $158^{\circ}$  melting took place, and continued heating caused decomposition. In the open air, the odor of burning sulphur was very noticeable when another test portion was heated to the decomposition point.

A third experiment was tried by using an aqueous solution of thioglycollic acid and bismuth oxide. The reaction proceeded very slowly and after several hours it was impossible to separate the acid salt; the decomposition was considerable. There was no doubt of a reaction having taken place.



However, the medium was not satisfactory for the isolation of a stable salt and, in all probability, one of the complex salts was formed. Neither an alkaline, an acid, nor even a neutral medium seemed conducive to the formation of a stable salt.

Salts of this composition have been reported:  $\text{Bi}(\text{SCH}_2\text{COOH})_3$ ;  $\text{Bi}(\text{SCH}_2\text{COO})_3$ ;  $\text{Bi}(\text{SCH}_2\text{COOH})_3(\text{HSCH}_2\text{COOH})_3 \cdot 5\text{H}_2\text{O}$ ;  $\text{Bi}(\text{SCH}_2\text{COOH})_3 \cdot 6\text{H}_2\text{O}$ .

Bismuth found, 43.11 per cent; calculated from formula  $\text{Bi}(\text{SCH}_2\text{COOH})_3$ , 43.44 per cent.

#### COPPER—SODIUM THIOLYCOLLATE

Cuprous hydrate was prepared by acidifying cuprous chloride and precipitating the yellow hydroxide by means of an excess of free fixed alkali. The excess of alkali was removed by repeated decantation, thus preventing the oxidation of the cuprous salt by keeping it constantly under the surface of the liquid. The suspended hydroxide was agitated with an excess of sodium thioglycollate solution. The reaction was quite rapid. The solution at first appeared canary yellow and, as the end point was reached, it became quite dark, indicating decomposition. More sodium thioglycollate was added and the yellow color reappeared. The solution was cooled in an ice mixture and the free acid salt was obtained by the addition of ten per cent sulphuric acid. Any other mineral acid causes decomposition. The moist free acid salt is yellow in appearance and decomposes upon standing in the open. The excess of sulphuric acid is removed by washing with water containing free thioglycollic acid. The salt is dried in vacuum. Alcohol cannot be used in this preparation as it hastens decomposition. The dry free acid salt becomes steel gray in appearance. The acid salt is not very soluble in water, however, the sodium salt dissolves quite readily but must be used almost immediately, on account of decomposition. The solution does not precipitate blood serum. Analyses showed that the salt contained 41.01 per cent Cu; calculated from the formula  $\text{CuSCH}_2\text{COOH}$ , 41.10 per cent. Sulphur calculated 20.09; found 19.95.

#### RUBIDIUM—SODIUM THIOLYCOLLATE

Sodium thioglycollate in solution was treated with a ten per cent solution of rubidium carbonate. Carbon dioxide was evolved and the solution became pink in color. By the addition of alcohol and ether a precipitate separated. This was dried in vacuum. The solution used was pink, but upon long standing it became a light yellow. The rubidium content was determined as the double chloride of platinum,  $\text{PtRb}_2\text{Cl}_6$ . Analyses showed that the salt contained 36.10 per cent Rb, calculated from the formula  $\text{RbSCH}_2\text{COONa} \cdot 2\text{H}_2\text{O}$ , 35.49.

#### SILVER—SODIUM THIOLYCOLLATE

The sodium salt of thioglycollate acid was treated with fresh silver oxide suspended in water. The first portion went into solution very readily and the solution became light yellow in appearance. With continued addition of the oxide, the solution became dark brown. The solution was filtered, to remove any solid particles, and then concentrated under diminished pressure.

The sodium salt was precipitated as an amorphous powder by means of absolute alcohol. It has a yellow appearance which changes, on standing a long time, to a grayish brown. The salt is readily soluble in water, but in solution gradually decomposes with the formation of silver sulphide. Analyses showed that the salt contained 48.60 per cent Ag; calculated from the formula  $\text{AgSCH}_2\text{COONa}$ , 48.82.

#### GOLD—SODIUM THIOGLYCOLLATE

Two experiments were carried out in the preparation of the gold salt. In one case the hydroxide was used and in the other the sodium gold chloride. The latter gave the better results. The sodium salt of thioglycollic acid in water was treated with a suspension of gold hydroxide. The first portions went into solution very readily with no color change. In the next stage, addition of more hydroxide caused a deep purple color to appear in a perfectly clear solution. Further addition causes a dirty green precipitate to form, and a still further addition gives a yellowish brown precipitate. This yellow precipitate could not be gold hydroxide, for the reason that gold hydroxide is soluble in an excess of fixed alkali. This precipitate was soluble in mineral acids, and was reprecipitated with the addition of alkali; it had a tendency to turn dark purple upon standing in the sunlight. It could not be obtained in crystalline form.

In the second preparation, sodium gold chloride was used. The sodium salt of thioglycollic acid was treated with sodium gold chloride with the formation of a light yellow precipitate. An excess of sodium thioglycollate was always present. The precipitate was filtered off, washed with ether and dried in vacuum. It turned a light brown color when dried. The salt was amorphous and readily soluble in water, forming a clear yellow solution which was very stable. Analyses showed that the compound contained 59.78 per cent gold. A compound of the composition  $\text{AuSCH}_2\text{COONa} (\text{H}_2\text{O})$  would contain 60.06 per cent Au; sulphur calculated 9.74, found 9.98. The salt must be well powdered after drying, and then it is necessary to macerate it in order to hasten solution. Addition of physiologic salt solution causes the salt to decompose, the solution becoming red, or almost similar to a colloidal gold solution. It is not advisable to use any chemicals to prevent the growth of molds as they likewise cause a decomposition.

#### BERYLLIUM (GLUCINUM)—SODIUM THIOGLYCOLLATE

Beryllium fluoride was warmed with hydrochloric acid until all of the fluorine was driven off. The liquid was evaporated to a small volume and slowly added to a solution of sodium thioglycollate. As the solution became less alkaline it assumed a light cherry red color. Further addition of an alkali caused a slight precipitate. The solution was filtered and concentrated under diminished pressure. Absolute alcohol precipitated an amorphous solid which was dried in a vacuum desiccator. The fact that no crystalline compound was formed, seemed conclusive evidence that there was a union between beryllium and sulphur; for the hydroxide of beryllium is soluble in an excess of fixed alkali, which would make it possible for sodium thio-

glycollate and the dissolved hydroxide of beryllium to coexist, and sodium thioglycollate would crystallize out. However, analysis seemed to verify the chemical combination, as well as the failure of a crystalline compound to form. Analysis showed that the salt contained 7.10 per cent Be; calculated from the formula  $\text{BeSCH}_2\text{COONa}$ , 7.45.

#### CADMIUM—SODIUM THIOLYCOLLATE

The sodium salt of thioglycollic acid in solution readily dissolved the hydroxide of cadmium with the formation of a clear yellow solution. The sodium salt of thioglycollic acid was kept in excess. The free heavy metal salt was obtained by precipitation with dilute hydrochloric acid. The free acid salt ( $\text{Cd}(\text{SCH}_2\text{COOH})_2$ ) is a crystalline compound, as shown by microscopic examination. The sodium heavy metal salt was obtained by concentrating the filtered yellow liquid under diminished pressure and precipitating with absolute alcohol. The alcoholic precipitate was a heavy oil which formed at the bottom of the separatory funnel. At  $60^\circ \text{C}$ . it remained viscous, but upon cooling a white crystalline sodium salt separated out. It was easily washed and then dried in vacuum. The compound is preserved very well in a vacuum desiccator but turns a little dark after long standing in the atmosphere. Upon further analysis and examination it was found that the probable formula for this salt was  $\text{Cd}(\text{SCH}_2\text{COONa})_2 \cdot 4\text{H}_2\text{O}$ . Analyses showed that the salt contained 27.79 per cent Cd; calculated from the formula  $\text{Cd}(\text{SCH}_2\text{COONa})_2 \cdot 4\text{H}_2\text{O}$ , 27.37. The salts reported by Rosenheim (1904) are  $\text{Cd}(\text{SCH}_2\text{COO})_2\text{Cd}$ ;  $\text{Cd}(\text{SCH}_2\text{COONa})_2 \cdot 3\text{NaCl} \cdot 6\text{H}_2\text{O}$ ;  $\text{Cd}(\text{SCH}_2\text{COO})_2\text{Ba} \cdot 18\text{H}_2\text{O}$ . The solution of cadmium is very stable over long periods. S found 15.84, calculated 15.62.

#### MERCURY—SODIUM THIOLYCOLLATE

Freshly prepared mercurous oxide is allowed to act upon a solution of sodium thioglycollate. The action is quite rapid, ten to fifteen minutes being necessary to complete the change. The first addition of suspended oxide gives a clear solution. Further addition causes a grayish white precipitate to separate out. The free acid salt is obtained by using a large excess of mineral acid. It is a needle-like formation. The sodium mercury salt is a crystalline compound obtained by precipitation with absolute alcohol. It is very soluble and very stable, except upon exposure to strong sunlight. Analyses of the free acid showed the salt to contain 52.39 per cent of mercury. A formula of the constitution  $\text{Hg}(\text{SCH}_2\text{COOH})_2$  requires 52.41 per cent. The solution is very stable but cannot be made up with physiologic salt solution. Claesson (1877) reports several mercury salts, one of which is the same as mentioned above. Other salts reported are  $\text{Hg}(\text{OOCCH}_2\text{S})_2\text{Hg}$ ,  $\text{Ag}_2(\text{OOCCH}_2\text{S})\text{Hg}$ , and complexes with no other metals.

#### THALLIUM—SODIUM THIOLYCOLLATE

Sodium thioglycollic acid is treated with thallic chloride with the formation of a light colored precipitate which finally goes into solution. An excess of thioglycollic acid is present. The solution becomes only slightly warm and then it is concentrated under diminished pressure. Absolute alcohol precipi-

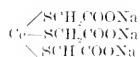
tates a fine needle-like compound, readily soluble in water to a perfectly clear solution, and very stable. The free acid salt decomposes into a brown amorphous powder. The sodium salt is more stable. Analyses showed the free acid to contain 43.17 per cent of thallium; calculated from the formula  $\text{Ti}(\text{SCH}_2\text{COOH})_3$ , it gives 42.74.

#### TITANIUM

The easy formation of orthotitanic and metatitanic acids renders the use of titanium rather difficult to work with. The fact that all titanium salts break down into one acid or another through hydrolysis, led to the selection of the sulphate in the attempt to prepare the thioglycollic acid salt. As in the case of tellurium, a dark precipitate was formed when the titanium salt was mixed with sodium thioglycollate. Ammonium sulphide produces orthotitanic acid in the cold and meta acid in the hot. From this fact it was concluded that the precipitate must be a combination of thioglycollic acid and titanium. When heated it gave the odor of sulphur, but upon treatment with acid it was impossible to obtain a qualitative test for thioglycollic acid. The salt was insoluble in acids and alkalis and consequently it was unsatisfactory for biological purposes. For this reason no further study was made of this preparation.

#### CERIUM—SODIUM THIOLYCOLLATE

The sodium salt of thioglycollic acid in solution is treated with cerous hydroxide  $\text{Ce}(\text{OH})_3$ . The solution is concentrated and the salt precipitated with alcohol and ether. It is washed with alcohol and ether, to shorten the period of drying as much as possible. The salt is then placed in a vacuum desiccator and finally well dried. The acid salt is an amorphous, white compound; while the sodium salt is crystalline. Cerium is determined as the oxide and as the oxalate. Analyses showed that the compound contained 29.53 and 29.39 per cent cerium. A formula which would correspond to this percentage composition, is



A compound of this constitution would require 29.26 per cent of cerium. Analyses showed it to contain 29.46 per cent. The solution keeps well for a few days, but after that a flocculent white precipitate appears. No salt of this metal is reported.

#### LEAD—SODIUM THIOLYCOLLATE

Freshly prepared lead hydroxide and sodium thioglycollate in solution were agitated in a closed flask. The first addition of lead hydroxide went into solution readily. After a short while the clear yellow solution began to solidify and became about the consistency of gelatin. This was very soluble in strong sodium hydroxide, and gave a clear solution which was precipitated by absolute alcohol. By dissolving this precipitate in water and adding a little alcohol, needle-like, shining, white crystals separated out. In the presence of moisture considerable difficulty was experienced on account of decomposition. A pure product was obtained in the end which proved to be a simple lead sodium salt.

The fact that lead hydroxide is soluble in excess of fixed alkali, forming the plumbate, makes it necessary to be cautious about adding too much hydroxide of lead. Lead sulphide is easily formed, and it may be said that the lead preparation is one of the most difficult to make. An excess of sodium thioglycollate is present. Analyses showed that the lead salt was probably the straight substitution product with two molecules of water of crystallization,  $\text{Pb}(\text{SCH}_2\text{COONa})_2 \cdot 2\text{H}_2\text{O}$ . Lead found, 45.50 per cent; calculated, 45.87. Salts reported are:  $\text{Pb}(\text{SCH}_2\text{COO})_2 \cdot \text{Pb}$  and  $\text{Pb}(\text{SCH}_2\text{COO})_2 \cdot 2\text{PbNa}_2 \cdot 2\text{H}_2\text{O}$ . The solution is very unsatisfactory for biological purposes.

#### VANADIUM—SODIUM THIOGLYCOLLATE

Sodium thioglycollate was treated with vanadic acid in large excess and the mixture warmed on a water-bath with occasional shaking for several hours (2 to 4). The medium was slightly alkaline, and the reaction was slow. At first the liquid was bluish, but upon further digestion it became green. The solution was filtered, to remove any excess of, or undigested, vanadic acid; it was again filtered and concentrated under diminished pressure. A blue, crystalline, sodium salt was obtained which was very stable. The solution is very satisfactory. Analyses showed that the compound contained 21.37 per cent V; calculated from the formula  $\text{V}_2(\text{SCH}_2\text{COON})_3 \cdot 2\text{H}_2\text{O}$ , 22.0.

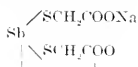
#### ARSENIC—SODIUM THIOGLYCOLLATE

Arsenious acid is added to a dilute solution of sodium thioglycollate and allowed to digest at about 40° C. for one day. The solution is filtered, concentrated under diminished pressure, and precipitated with absolute alcohol and ether.

Arsenious chloride is used with free thioglycollic acid dissolved in water and the resulting solution is just neutralized with a solution of sodium carbonate. Sodium chloride is removed in the manner described under antimony. The resulting products in these two experiments were the same, as shown by their crystal formation under the microscope. The microscope was found very serviceable in examining the crystalline salts in this investigation; as it was thereby quite easy to detect impurities. Analyses showed that this compound contained 17.20 per cent As; calculated from the formula  $\text{As}(\text{SCH}_2\text{COONa})_3 \cdot \text{H}_2\text{O}$ , 17.34.

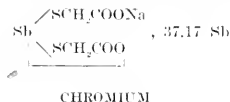
#### ANTIMONY—SODIUM THIOGLYCOLLATE

The antimony salt was prepared by treating antimony chloride with a solution of sodium thioglycollate. The solution was filtered and concentrated under diminished pressure until the sodium chloride separated out and, by microscopic examination, the complete separation was determined. The clear syrupy mixture was now treated with absolute alcohol, giving the compound



Free thioglycollic acid was dissolved in water and treated with antimony oxide, by warming the solution on the water-bath. After standing for twenty-four

hours, the solution was filtered and treated with sodium carbonate, concentrated under diminished pressure, and precipitated with absolute alcohol and ether. It is found that in this case alcohol and ether give better results. Analyses showed that this compound contained 36.80 per cent Sb; calculated from the formula



All attempts to prepare the chromium salt failed. Chromium chloride was treated with the sodium salt of thioglycollic acid with the formation of chromium hydroxide. The hydroxide was used with no perceptible action even after several days. Chromium oxide was then tried with similar results. The final attempt was made with chromium sulphate, which likewise failed. That no one may be misled in his efforts, it should be stated that the author makes no claim that this salt cannot be prepared. Time was not available to make a detailed study of the constitution of these preparations, or to prepare those which withstood the ordinary methods of preparation.

#### MOLYBDENUM—SODIUM THIOLYCOLLATE

Sodium thioglycollic acid in solution is treated with molybdenum trioxide suspended in water. An immediate reaction takes place, with the formation of a steel-gray precipitate which is very soluble in water. The precipitate is filtered off, washed with ether and dried in vacuum. The salt dissolves very readily in a neutral solution, is crystalline and has a grayish appearance. The keeping qualities of the dark, reddish brown solution are very good. Molybdenum seems to enter into combination very easily and is in accord with the thiomolybdate formation. Analyses showed this compound to contain 15.97 per cent Mo. Calculated from the formula  $\text{Mo}(\text{SCH}_2\text{COONa})_4 = 16.43$ .

#### TELLURIUM

Sodium tellurate was mixed with sodium thioglycollate with the formation of a precipitate. The precipitate was filtered off and treated with fixed alkali, water and acid. In each case the compound showed no signs of solution. Qualitative tests showed that the precipitate was a sulphide of tellurium.

#### TUNGSTEN—SODIUM THIOLYCOLLATE

Sodium thioglycollate was treated with a suspension of tungsten oxide and allowed to digest for two days. The solution had a green color and was concentrated under diminished pressure. Upon long standing, greenish yellow plates separated out; these were very soluble in water. The dry salt was yellow. Analysis showed 20.1 tungsten, and the calculated value for the formula  $\text{W}(\text{SCH}_2\text{COONa})_6 \cdot 2\text{H}_2\text{O}$  would be 20.59.

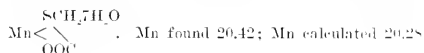
#### URANIUM—SODIUM THIOLYCOLLATE

Sodium thioglycollate was treated with a dilute solution of uranyl chloride. A yellowish green solution was formed. The mixture was allowed to di-

gest for twenty-four hours, it was then filtered and concentrated under diminished pressure. Yellowish green crystals separated out, which were filtered and dried. The solution is very unstable, and the solid is readily soluble in water. The uranyl salt is unsatisfactory for biological purposes. Analysis showed it to contain 41.20 per cent U; calculated from the formula  $\text{UO}_2(\text{SCH}_2\text{COONa})_2 \cdot 4\text{H}_2\text{O}$ , 41.91.

#### MANGANESE—SODIUM THIOGLYCOLLATE

Manganous hydroxide was prepared and the white precipitate washed by decantation to prevent oxidation. The sodium salt of thioglycollic acid, dissolved in water, is treated with successive additions of the suspended hydroxide. The reaction begins immediately and a pink solution is formed. However, there is a tendency toward oxidation, as indicated by the traces of brown coloration of the manganese hydroxide. By concentration under diminished pressure the manganese salt is crystallized out as prisms. Upon drying, this salt and making a solution, one notices a pinkish color indicative of a manganous salt. Upon standing, the solution turns a yellowish brown. A change in toxicity is noticed with this action. It was impossible to dry the salt in the air without decomposition; there was some change also in solution. It was concluded that the salt was in the manganous condition. Rosenheim (1904) reports a salt of the formula:

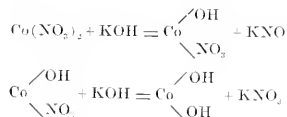


#### NICKEL—SODIUM THIOGLYCOLLATE

The suspended nickelous hydroxide is added in small portions to sodium thioglycollate in solution, which at first is pale green. The solution is concentrated under diminished pressure and greenish brown prisms separate from the solute. The salt, when dissolved in water, has a dirty green color which completely disappears upon standing. Absolute alcohol precipitates the sodium salt. Mineral acids dissolve the salt with the formation of an almost colorless solution. The nickel salt gives a very poor solution for biological purposes. Analyses showed it to contain 16.70 per cent of nickel; calculated from the formula  $\text{Ni}(\text{SCH}_2\text{COONa})_2 \cdot 4\text{H}_2\text{O}$ , we obtain 16.53 per cent of nickel. This is in accordance with the investigations of Rosenheim (1904).

#### COBALT—SODIUM THIOGLYCOLLATE

Cobaltous hydroxide was prepared by precipitating the nitrate with potassium hydroxide:



The blue basic salt was first formed and was heated to boiling to prevent the formation of the cobaltoso-cobaltic hydroxide, which is olive green, while the pure

cobaltous salt is pink. The precipitate is washed free of alkali and treated with a solution of sodium salt of thioglycollic acid. At first, there is a blue, then a pink, and, finally, a dark brown coloration appearing in the mixture. By gently warming the mixture, the pink color returns. The solution is filtered and concentrated as previously described. Absolute alcohol and ether are used to precipitate the salt which is dried as usual. It is very soluble in water. It is a dark red, crystalline compound, the crystals having a columnar form under the microscope. The cobalt compounds which have been reported are:  $\text{Co}(\text{SCH}_2\text{COO})_2$ , and  $\text{Co}(\text{SCH}_2\text{COONa})_2 \cdot 6\text{H}_2\text{O}$ . The compound obtained corresponded with this last formula, but the tendency toward decomposition was very marked. A precipitate formed, when the substance was dissolved in water, which still seemed to be a thioglycollic acid compound, as indicated by heating the product and by solution in mineral acids which gave the characteristic color reaction. The keeping qualities of the cobaltous solution were very poor. Analyses showed that it contained 15.15 per cent Co; calculated from the formula  $\text{Co}(\text{SCH}_2\text{COONa})_2 \cdot 6\text{H}_2\text{O}$ , 15.01 per cent.

#### PLATINUM—SODIUM THIOLYCOLLATE

Sodium thioglycollic acid was treated with dilute platinum chloride. At first the solution became yellow, then red and, with further addition, a yellowish brown precipitate formed which was filtered off and washed with absolute alcohol. A compound, very light yellow in color and very stable, results. The salt is very soluble in water; as much as thirty milligrams of metallic platinum per cubic centimeter can be dissolved. In the concentrated solutions, the appearance is distinctly red, fading to a light yellow in the more dilute. The free acid was very similar in appearance, but not as soluble in water. Analyses of the free acid showed the following results: Pt 34.60 and 34.64; calculated according to the formula  $\text{Pt}(\text{SCH}_2\text{COOH})_4$ , 34.88. The sodium salt gave 30.22 platinum and the calculated value for  $\text{Pt}(\text{SCH}_2\text{COONa})_4$ , 30.14. No record of a platinum salt was found. Claesson (1906) says the salt  $\text{Pt}(\text{SCH}_2\text{COO})_2$  may be prepared from potassium chlor-platinate.

#### ZINC—SODIUM THIOLYCOLLATE

Zinc oxide and sodium thioglycollate were allowed to react, yielding a slightly yellow solution. Acids throw out a white crystalline material, which is soluble with difficulty in water but easily goes back into solution upon the addition of alkalis. The final product was a crystalline material very easily dissolved in water. Analyses showed that the compound contained 20.83 per cent Zn. Calculated from the formula  $\text{Zn}(\text{SCH}_2\text{COONa})_2 \cdot \text{H}_2\text{O}$ , 21.11.

NOTE: In some cases the heavy metal salt of thioglycollic acid was used for analysis. In every case the composition of the salt used for analysis is given.

These preparations were all tested for toxicity upon rats and guinea pigs. The trypanocidal action was also tested on rats and guinea pigs and a report of these tests will be found in a paper soon to be published by Dr. G. C. Lake of the U. S. Hygienic Laboratory. Antimony potassium lactate was also included in this series and found to be highly active by Dr. Lake. Further reports may be found in the work of Voegtlin and Smith.



## ACKNOWLEDGMENT

I wish to here express my sincere appreciation to Dr. Carl Voegtlin for his kindly interest and suggestions in carrying out this investigation in the Division of Pharmacology.

PERIODIC ARRANGEMENT OF METAL SALTS USED IN THIS INVESTIGATION

	0	I R <sub>2</sub> O	II RO	III R <sub>2</sub> O	IV $\frac{RH_4}{RO_2}$	V $\frac{RH_3}{RO}$	VI $\frac{RH_2}{RO}$	VII $\frac{RH}{RO}$	VIII
1	-	-	-	-	-	-	-	-	-
2	-	-	Gl(Be) 9.1	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-
4	-	-	-	-	Ti 48.1	V 51.0	Cr 52.0	Mn 54.93	Ni, Co 58.68, 58.97
5	-	Cu 63.57	Zn 65.37	-	-	As 74.96	-	-	-
6	-	Rb 85.45	-	-	-	-	Mo 96.0	-	-
7	-	Ag 107.88	Cd 112.4	-	Sb 118.7	Sb 120.2	Te 127.5	-	-
8	-	-	-	-	Ce 140.25	-	-	-	-
9	-	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	W 184.0	-	-
11	-	Au 197.2	Hg 200.6	Tl 204.0	Pb 207.2	Bi 208.0	-	-	Pt. 195.0
12	-	-	-	-	-	-	U 238.2	-	-

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# A NOTE ON THE RELATION BETWEEN THE BLOOD-COAGULATING AND THE SMOOTH MUSCLE-CONTRACTING PROPERTIES OF TISSUE EXTRACTS \*

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THE action of tissue extracts, when injected intravenously into animals, has long been a subject of study. The toxic effects observed have been ascribed mainly to two causes: (1) the coagulant action of the extracts on the blood, and (2) the presence in the solutions of a substance which acts on smooth muscle. Many workers have limited their observations to only one of the above phases of tissue extract toxicity, ignoring or not recognizing the presence of the other factor. It would be inadvisable to attempt a review of all the mass of literature on this question, so mention will merely be made of a few of the papers. Wooldridge<sup>1</sup> was the first to notice the blood-clotting action of tissue extracts *in vivo*, and to make any extensive study of this action. Dold and his co-workers<sup>2</sup> made many observations along this line also. (For references to literature see Smith<sup>3</sup> and Mills.<sup>4</sup>) As regards the smooth muscle-stimulating substance, reference should be made to Brieger and Uhlenhuth,<sup>5</sup> Aronson,<sup>6</sup> Popielski,<sup>7</sup> Dale, et al.,<sup>8</sup> Abel and Kubota<sup>9</sup> and Smith.<sup>3</sup> Popielski's vasodilation possessed properties very similar to the histamine investigated so thoroughly by Dale and his co-workers, but was not at that time supposed to be identical with it. Abel and Kubota claim to have found histamine in many tissue extracts, and also a histamine-like body giving the imidazol test. This last they investigated especially in extracts of the pituitary gland. The smooth-muscle stimulating substance, or substances, are not regarded as having any influence on blood coagulation by most of these investigators, although by some it is thought that a slight inhibitory action is produced.

The possibility of the stimulation of the smooth musculature of the body by the intravascular clotting of the blood has not been much investigated. It is well known that certain sera, especially when fresh, are quite toxic for many animals, whereas the unclotted blood may be much less poisonous. A very good review of the literature regarding the toxicity of sera will be found in an article by DeKruif<sup>10</sup> on this subject. Schultz<sup>11</sup> found that smooth muscle from various parts of the body, when treated with serum *in vitro*, was stimulated to contract. The fresh blood, unclotted, had little or no such action, but as evidences of clotting appeared, the stimulating action became marked. Stewart and Zucker<sup>12</sup> and others (O'Connor,<sup>13</sup> Battelli,<sup>14</sup> Schultz<sup>11</sup> have also noted the stimulating action of serum on isolated smooth muscle

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strips, although Stewart and Zucker thought plasma possessed the same stimulating action, except on arteries. The evidence, then, points to the presence in serum of some substance not present as such in the whole blood, or else present in a combined and inactive form, from which it may be liberated by the clotting process.

The fact that in anaphylactic shock the smooth musculature of certain organs is very greatly stimulated and the blood pressure greatly reduced (see Richet,<sup>15</sup> Biedl and Kraus,<sup>16</sup> Pearce and Eisenbrey,<sup>17</sup> Edmunds,<sup>18</sup> Auer and Lewis,<sup>19</sup> Schultz,<sup>11</sup> Manwaring,<sup>20</sup> and others) brings this phenomenon in close relation to the effects observed after tissue extract or serum injection. It is evident that in anaphylaxis these phenomena cannot be due solely to the injection of histamine contained in the protein antigen, for the anaphylactic shock can be produced only a single time, while repeated injections of histamine may each show the typical action of the drug. This point was emphasized by Pelz and Jackson<sup>21</sup> in their study of the bronchiole constriction in dogs during anaphylactic shock. That anaphylactic shock is in some way related to the clotting of the animal's blood is indicated by the change in the coagulability of the blood during and following the shock. The subsequent negative phase or partial noncoagulability of the blood is much similar to the condition which frequently follows tissue extract injection (see Wooldridge.<sup>22</sup>) This fact, together with the observations regarding the presence of a toxin in serum not present in the unclotted blood, render necessary a close study of the effects of intravascular clotting of the blood and its bearing on anaphylactic shock.

The present work is concerned mainly with determining the effects of various fractions of tissue extracts, intravenously injected into dogs, in an effort to find the relationship between the smooth-muscle stimulating and blood clotting substances present. Intravascular clotting induced by the coagulant fraction, freed from the histamine or histamine-like substance, was studied in particular in relation to the effect of such clotting on the various involuntary organs of the body. The lungs, uterus, bladder and blood vessel system were the parts observed most closely.

The experiments herein described have been performed on dogs which had been etherized, prepared for the experiment, and then, in about half of the experiments, pithed (usually both brain and cord).

The uterus (*in situ*) tracings were made by means of a special device<sup>23</sup> which was placed inside the abdomen and held securely in position while the contractions of the organ were recorded by means of a metal hook attached to the uterus. A small string, fastened to the hook, passed over a system of pulleys and was finally attached to a light, counterpoised lever which marked the contraction on a smoked drum. The bladder tracings<sup>24</sup> were made by tying a large cannula into the fundus of the organ and connecting the upper end of the cannula to the tube of a mercury bulb. The bulb was then filled about half full of warm salt solution (to dilate the bladder) and a tube passing through the cork in the mercury bulb was connected to a recording tambour. The air in the upper half of the mercury bulb was thus connected by the

rubber tubing with the interior of the tambour bowl. The bronchiole tracings were made by means of a special method<sup>25</sup> by which air was intermittently aspirated from the chest cavity while the amount of air passing into and out of the lungs was indicated by means of a tambour connected with the side tube of the tracheal cannula. When the bronchioles were fully relaxed and the lungs became widely inflated each time air was aspirated out of the chest,

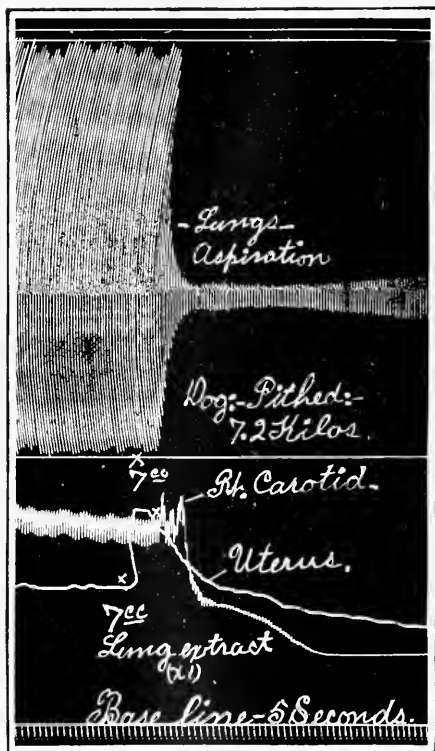


Fig. 1.

the tambour pointer moved down to a low level on the drum, and, as air was permitted to enter the chest cavity between the moments at which aspiration occurred, then, as the elasticity of the lungs caused their own collapse and forced air out through the trachea, the writing lever of the tambour was forced up to a high level. Contraction of the bronchioles decreased the amount of air passing into or out of the lungs and hence the tambour record on the drum at once decreased in amplitude. Profound bronchiole contraction might cause a complete cessation of the passage of air

into or out of the lungs, the force of aspiration of the air suction from the vacuum pump remaining constant. In these cases the tambour tracing became greatly reduced in amplitude, or perhaps marked only a straight line on the drum. Injections of extracts were usually made from a burette into a femoral

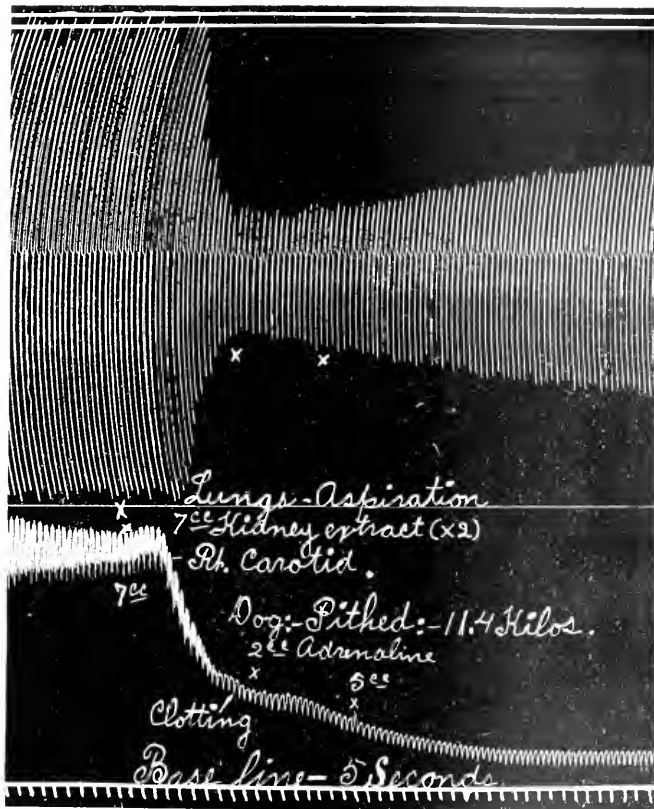


Fig. 2.

or a jugular vein. Blood pressure was recorded by a mercury manometer connected with a carotid artery.

The following extracts and solutions were used for the injections: (1) extracts of fresh normal tissues, mainly lungs, (2) crude antithrombin solution, (3) albumins of lungs extract, (4) purified antithrombin (globulin), (5) purified active coagulant (globulin) of lung extract, and (6) the purified

coagulant, inactivated. Each of these solutions will now be described more in detail, together with the effects produced by intravenous injection in dogs.

#### (1) EXTRACTS OF FRESH NORMAL TISSUES

These extracts were made by grinding the fresh tissue to a paste with

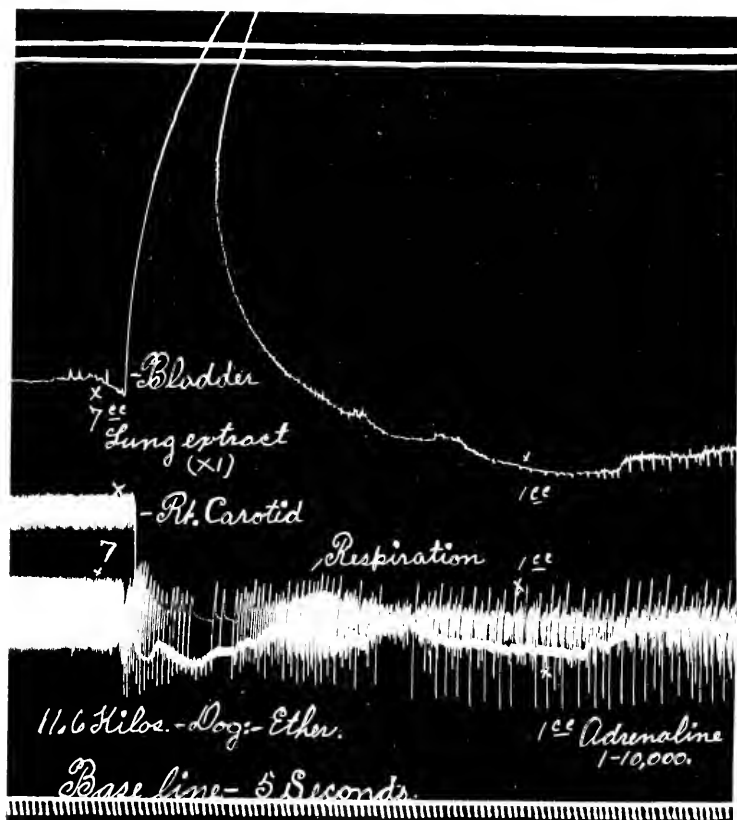


Fig.

white sand and extracting with 0.9 per cent NaCl solution in the ratio of 10 c.c. saline solution for each gram of fresh tissue taken. Lung extracts were used mainly because of their efficacy in inducing intravascular clotting. These extracts give a positive test for imidazol compounds, as tested by Ehrlich's diazo reaction. No effort was made to determine whether it was free histamine that was responsible for the positive imidazol test. The work of Abel

and Kubota indicated that free histamine was present in such extracts. Lung extract thus prepared contains approximately 0.74 per cent protein. Fig. 1 shows the action of 7 c.c. of this lung extract on the bronchioles, blood pressure and on the uterus *in situ*. The smallness of the dose is significant here. The animal was pithed (brain and cord) so that the actions shown in the tracing are entirely of peripheral origin. It will be noted that the right carotid tracing shows that extensive intravascular clotting occurred within a few seconds after the extract was injected. This, of course, caused immediate stoppage of the circulation, and at the same time a contraction of the bronchioles and of the uterus occurred.

Fig. 2 shows a quite similar action which occurred in a larger dog following the injection of 7 c.c. of kidney extract (but this extract was twice as strong as the preceding lung extract).

Fig. 3 shows the action of 7 c.c. of lung extract on the respiration, blood pressure and bladder in an intact, etherized dog. A marked contraction of the bladder occurred following the injection of the extract. At the same time the blood pressure fell to about half its former height. This was due to a rather extensive intravascular clotting of the blood, but in this particular case the heart was still able to go on beating feebly, and to maintain a very low and uncertain blood pressure as indicated by the mercury manometer. This was evidently possible because the clots formed had not completely filled all the important blood vessels in the heart and in the medulla, for the animal continued to carry on a weak, irregular respiration. It is, perhaps, a matter of some significance that a large dose of adrenalin injected later had only a very slight influence on the blood pressure.

## (2) CRUDE ANTITHROMBIN SOLUTION

It has been shown elsewhere by one of us (Mills<sup>4</sup>) that the active coagulant as it exists in the lung tissue is a protein phospholipin compound having the solubility characteristics of a globulin, and comprising practically the entire globulin content of lung extracts as made here. Analysis showed this to be composed of one protein molecule united to about 13 phospholipin molecules (probably cephalin). Removal of the phospholipin in a manner so as to leave the protein soluble destroys the coagulant action of the compound and leaves the protein fraction with antithrombic properties comparable to those of hirudin. Such phospholipin extraction can best be carried out by using benzene at room temperature until all available phospholipin has been removed. If fresh calf lungs be hashed, dried rapidly at room temperature, powdered and extracted by benzene in this manner, a saline extract (0.9 per cent NaCl) of this benzene extracted lung will no longer cause clotting of the blood *in vivo* or *in vitro*, but acts as an active anticoagulant. It is this anticoagulant lung extract that is here termed crude antithrombin. It contains 0.93 per cent of protein consisting of the soluble albumins of the lung and the globulin fraction of the active coagulant. Ehrlich's diazo reaction is positive, just as in the extracts of fresh normal lungs. This extract permits us to study the effects of tissue extracts in the absence of intravascular clotting.

Fig. 4 shows the result of injecting 30 c.c. of crude, antithrombin solution

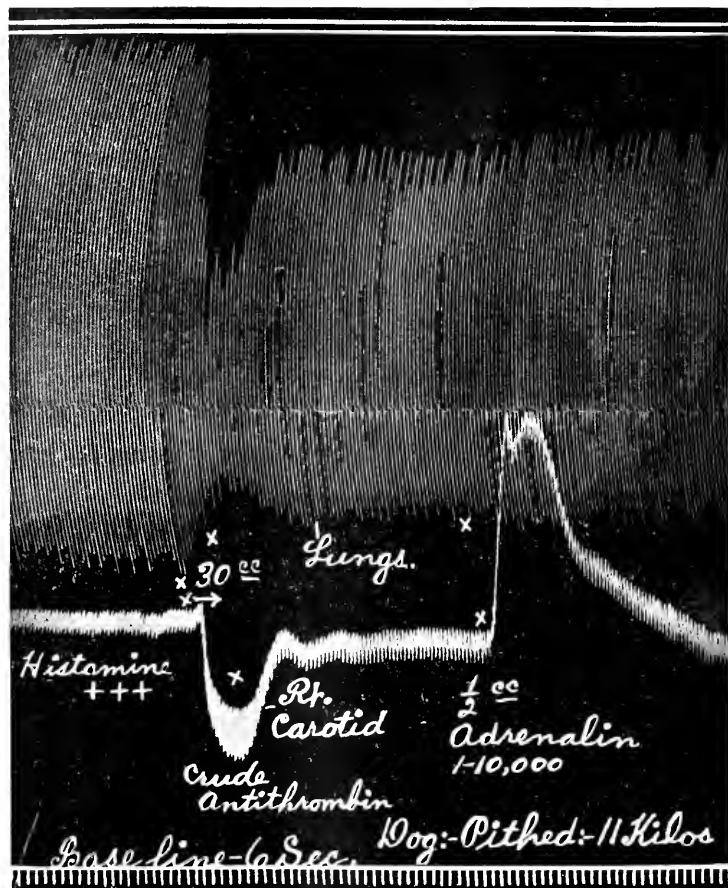


FIG. 4

into a pithed dog. No clotting occurs, yet the bronchioles contract and the blood pressure shows a marked fall. These actions we presume are due to histamine, or histamine like (Abel and Kubota) substances present in the extract as shown by the positive Ehrlich reaction.

#### (3) ALBUMINS OF LUNG EXTRACT

The solution containing (0.16 per cent protein) the albumins was obtained by precipitating the globulin fraction at its isoelectric point (about  $P_H = 5-6$ )



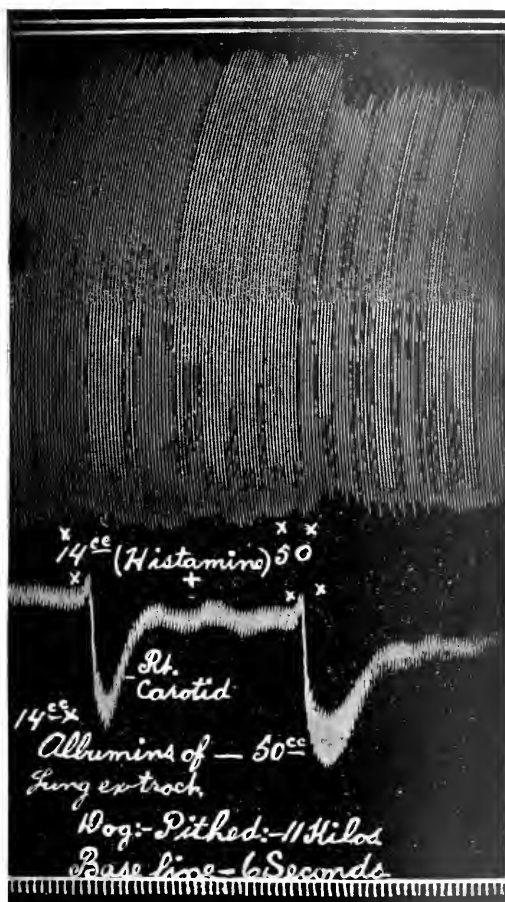


Fig. 5.

with  $\text{H}_2\text{SO}_4$ . The total globulin content was removed in this way, and the supernatant liquid containing the albumins, after neutralization with  $\frac{\text{N}}{2}$   $\text{NaOH}$ , was used for the injections. Albumins prepared in the same way from the crude antithrombin solution (saline extract of benzene extracted lung) were used in some experiments, but the effects were the same as with the albumins prepared from extract of fresh lungs. These albumin solutions gave a positive test with Ehrlich's diazo reaction for imidazol compounds. They are without action on the coagulability of the blood.

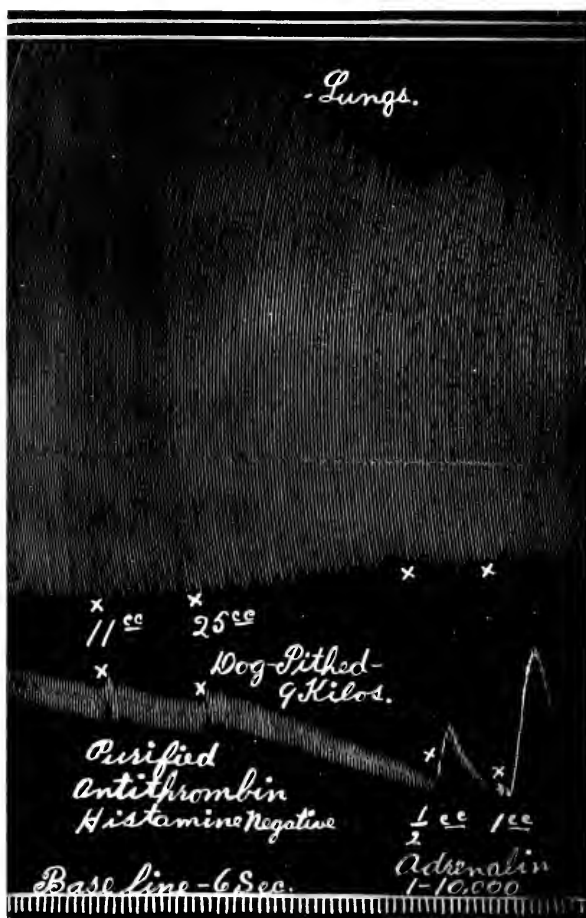


FIG. 5.

Fig. 5 shows the action of the albumins on the bronchioles and blood pressure of a pithed dog. The fall in blood pressure and contraction of the bronchioles we believe to be due to the presence of histamine, or histamine-like, bodies as was described in the case of the crude antithrombin. (The bronchiole contraction is not as marked here as it often occurs after the injection of the albumins, because the negative air pressure used to aspirate the chest cavity was slightly stronger than it should have been.)

## (4) PURIFIED ANTITHROMBIN SOLUTION

The anticoagulant globulin was purified by precipitating from the crude antithrombin solution at its isoelectric point and washing the precipitate in distilled water by decantation until the imidazol ring test was entirely negative. Solution in 0.9 per cent NaCl solution was then effected by adding a slight amount of  $\frac{N}{2}$  NaOH. This globulin solution, free of all traces of imidazol ring compounds, was used for the injections. The use of acid in its purification largely destroys its anticoagulant properties, so that the solution is almost inactive as regards blood clotting. To retain the active anticoagulant action salt precipitation must be used in the purification. This yields a final product which possesses the same intravenous effects as the acid precipitate. The isoelectric precipitation was used because of the speed with which a pure product can be obtained. The purified antithrombin solution used contained approximately 0.43 per cent of protein.

Fig. 6 shows the effects produced when the purified antithrombin is injected intravenously. The sharp, abrupt fall of blood pressure, which characterizes the action of histamine, or histamine-like bodies is entirely absent as is also the marked, sudden bronchiole contraction. There is, however, produced a slow, gradual bronchiole contraction, and this may be due to the specific action of the antithrombin itself, as may also be true of the slow, gradual fall in blood pressure (which in this case seems very probably to be due to a weakening of the heart).

## (5) PURIFIED ACTIVE COAGULANT SOLUTION

The active coagulant may be purified exactly as was the anticoagulant. However, it does not lose very much of its activity by precipitation at its isoelectric point, so this method was used entirely, an active product entirely free from imidazol ring compounds being obtained. This solution readily caused clotting *in vivo* or quickened clotting *in vitro*. It could not possibly contain any histamine or histamine-yielding constituent since the imidazol ring was entirely absent. The same holds true for the pure anticoagulant.<sup>5</sup> The purified active coagulant was made by precipitating the active globulin from lung extract by acid ( $N$  500  $H_2SO_4$ ), washing the precipitate and redissolving it in 0.9 per cent NaCl solution by the addition of a small amount of alkali. It contained approximately 0.23 per cent of protein.

Fig. 7 shows the action of the active coagulant on the bronchioles and blood pressure when injected intravenously. Intravascular clotting occurred at once, and the bronchioles were profoundly contracted. Since this active coagulant contained no imidazol ring compounds we believe the action on the bronchioles was produced by some substance which is set free in the body as the blood coagulates. This same type of action may be present in other tissues (as muscles, etc.) also, but we do not wish to discuss that point at present. We are of the opinion that as the blood coagulates, it sets free the substance which acts on the smooth musculature, and we strongly suspect that this may be histamine or some very nearly related substance. The action

on smooth muscle here is absolutely typical for histamine. And the indication shown in Fig. 3 that, after injection of lung extract and extensive intravascular clotting, adrenaline seemed to have but little power to raise blood pressure, is in harmony with what we might expect after a marked histamine reaction in the capillaries and minute arteries and veins.



Fig. 7

#### (6) PURIFIED COAGULANT, INACTIVATED

A portion of the solution of purified active coagulant was treated with  $\frac{N}{2}$  NaOH to give a calculated alkalinity of about  $\frac{N}{15}$  and the solution boiled. No coagulation took place, alkaline metaprotein probably being formed by the

action of the NaOH. The coagulative activity of the compound on blood was almost completely destroyed by this treatment. Neutralization back almost to neutrality with  $\frac{N}{2}$   $H_2SO_4$  gave an inactive solution of the pure coagulant.

Fig. 8 shows the action of two different solutions, first that of the purified coagulant which has been inactivated as described above. Here an injection

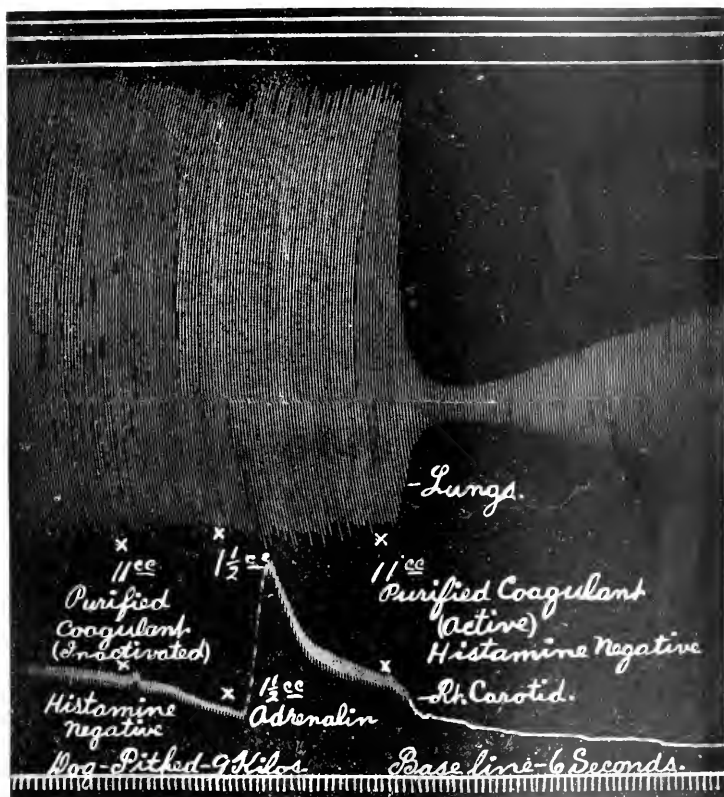
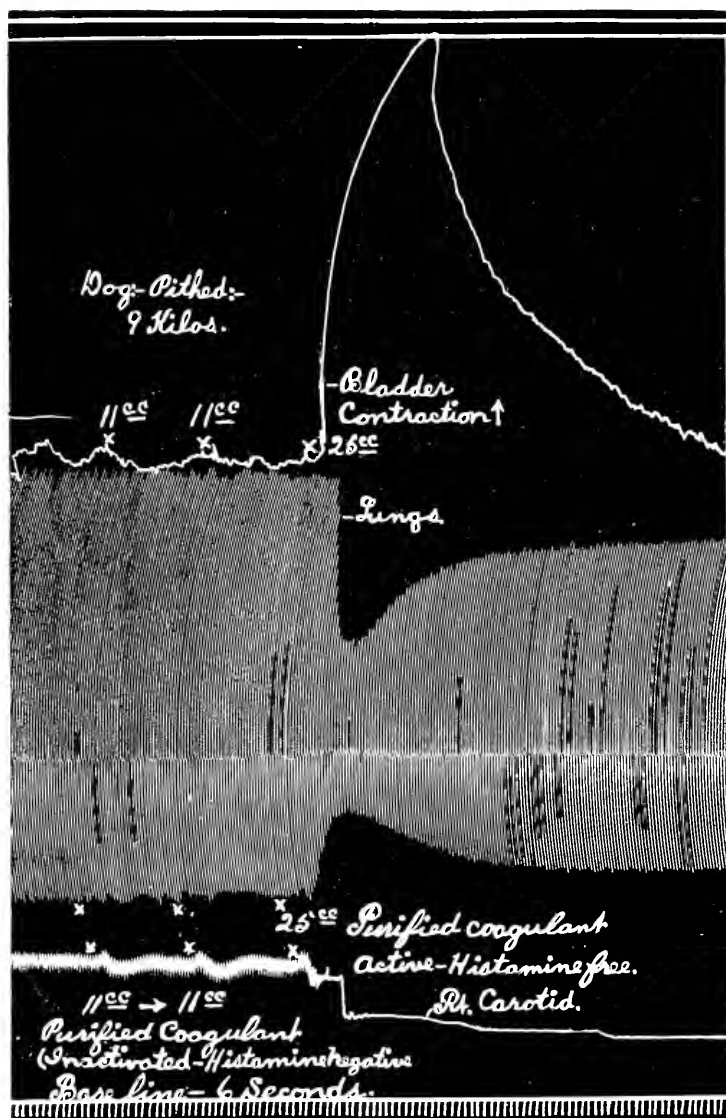


Fig.

of 11 c.c. had practically no effect on the bronchioles, and the action on the blood pressure in no wise resembled that of histamine, neither did intravascular clotting occur. The heart, however, was weakened considerably, and to overcome this, an injection of  $H_2$  c.c. of adrenalin solution was given. This strengthened the heart and raised the blood pressure. Following this a third injection was given. This consisted of 11 c.c. of the active coagulant. A



prompt intravascular clotting of the blood occurred, and at the same time the bronchioles contracted vigorously. These reactions occur after atropin and are therefore muscular in origin.

Fig. 9 shows these same points again, but, in addition, the effects on the bladder are also illustrated. In the beginning of this tracing two separate injections of 11 c.c. each of purified coagulant *inactivated* were given. These produced no effects on either the bladder or lungs, and the slight variations in the blood pressure were due, perhaps almost solely, to the sudden addition of the injected solution to the whole volume of the blood in the animal's vascular system. This solution had simply been heated, after the addition of a small amount of alkali, and then brought back as nearly as possible to the point of neutrality by the addition of very weak acid. The third injection in the tracing shows the effects produced by 25 c.c. of the active coagulant. All three of these injections were made from the same original solution, but in the first two (11 c.c., 11 c.c.), a portion of the extract had been inactivated. No histamine, or histamine-like substance, was present in either solution. But one had no effect on the blood, while the other produced immediate intravascular clotting. And when no action on the blood occurred, no contractions of the smooth musculature (or fall of blood pressure) occurred, while, when the blood was made to clot, very marked contractions of both the bladder and the bronchioles occurred. The very striking similarity of these smooth muscle reactions to those produced in anaphylactic shock is obvious at a glance. We do not doubt but that the two phenomena are exceedingly closely related, if indeed, they are not identical in their fundamental nature. We have performed corresponding experiments with the purified coagulant and with the purified coagulant inactivated on isolated strips of the guinea pig's uterus *in vitro*. The results coincide exactly with the findings above described, contraction of the uterus occurring just as the (citrated horse) plasma is made to coagulate by addition of active coagulant and calcium chloride. Essentially this same observation has been made by others, especially Schultz, Stewart, etc., on the action of whole blood, just as it coagulates (naturally) on the guinea pig's uterus *in vitro*, or on the action of serum from blood clots on isolated strips of smooth muscle. We presume that any substance which will cause rapid intravascular clotting of the blood, will produce these smooth muscle contractions, if injected suddenly in sufficient quantity into the circulation.

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## OBSERVATIONS ON THE PHARMACOLOGY OF SOME BENZYL ESTERS\*

BY CARL NIELSEN AND JOHN A. HIGGINS, CHICAGO, ILL.

WITHIN the past few years, David I. Macht<sup>1</sup> has published various articles pertaining to the pharmacology of benzyl esters and the benefits derived from their use in medicine. His careful investigations were based upon his discovery of the relation, in chemical structure and pharmacologic action, between the papaverin group of the opium alkaloids and these benzyl esters. His interesting findings have naturally caused the medical profession to study one of these benzyl esters (benzyl benzoate) clinically and a number of reports on its beneficial action, in a variety of clinical conditions, have since appeared, all based upon the property of benzyl benzoate to relieve spasmodic pain.

We have for some time been conducting investigations of the pharmacologic action of a series of benzyl esters, some of them liquids, others solids, including the benzyl benzoate as originally recommended by Macht. While our work has been in progress, an article has appeared in this journal, published by Mason and Pieck,<sup>2</sup> and, though we are not prepared at this time to give all the data we have obtained but shall publish our main report at a later date, yet we feel inclined to present in this preliminary paper the results of our experiments with benzyl benzoate on the intestine *in situ*, as well as those obtained with benzyl cinnamate.

The benzyl esters under investigation have been supplied in chemically pure form by the chemical research department of the Abbott Laboratories. In testing these, we have experienced the same difficulties as other investigators, especially as regards a suitable means of diluting the benzyl esters for intravenous injections. In our study of the action of these benzyl compounds on the intestines, we have found emulsions of the benzyl compounds

\*From the Pharmacologic Department of The Abbott Laboratories, Chicago, Ill.



made with gum acacia to be the most favorable for intravenous administration. These emulsions were prepared in such a manner as to contain the smallest possible amount of gum acacia to assure thorough distribution of the benzyl compounds. We have, however, also used alcoholic solutions of the esters in such instances where the alcohol could be proved not to interfere too greatly with their actions.

Using a method as described by Jackson<sup>2</sup> we have been able to obtain marked intestinal relaxation following injections of relatively small amounts

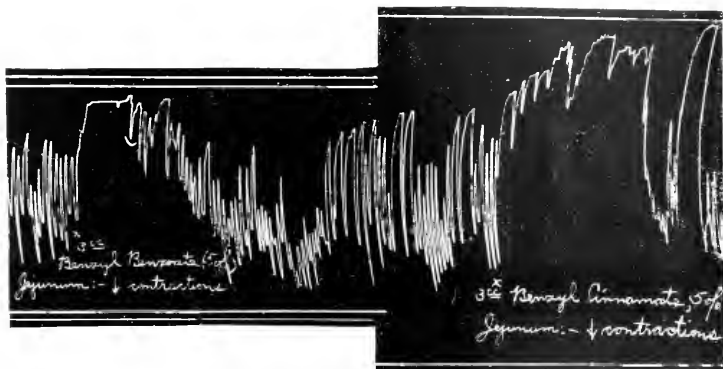


Fig. 1. (For description, see text.)

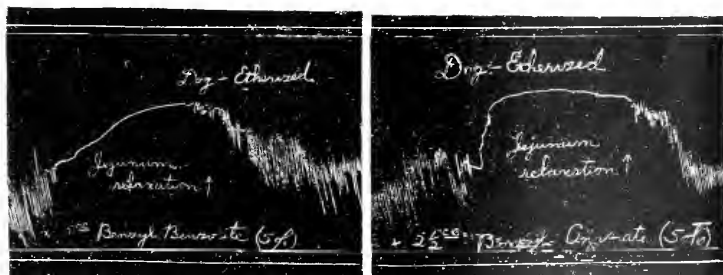


Fig. 2. (For description, see text.)

of benzyl benzoate and benzyl cinnamate (Figs. 1 and 2). Experience has taught us the necessity of great care in preparing the animal and also of patience in waiting for the benzyl compounds to be absorbed and to exercise their action, and allow the intestine to return to normal contractions before another injection is made. It will be noted that the benzyl cinnamate produced a greater intestinal relaxation than benzyl benzoate in equal amounts. In verifying this point, we were able to relax the intestine to a greater extent with only one-half the amount of benzyl cinnamate.

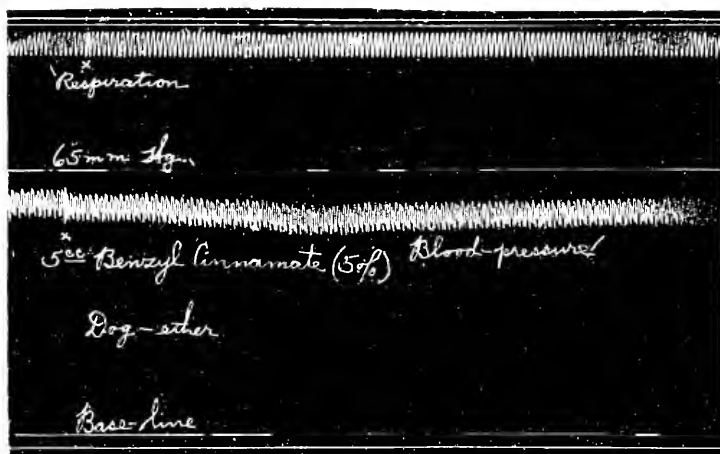


Fig. 3A. (For description, see text.)

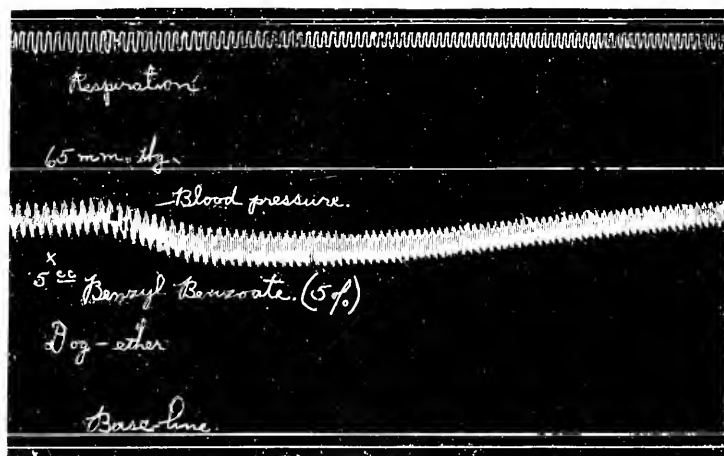


Fig. 3B. (For description, see text.)

Our records of the blood pressure (Fig. 3) seem to indicate that benzyl cinnamate lowers the blood pressure less than benzyl benzoate.

From the above, it will be seen that our findings do not harmonize with those of Mason and Peck. These authors report a negligible effect, or no effect at all, on the intestine. They used a somewhat different method (finger cot) and injected barium chloride in order to stimulate the movements of the

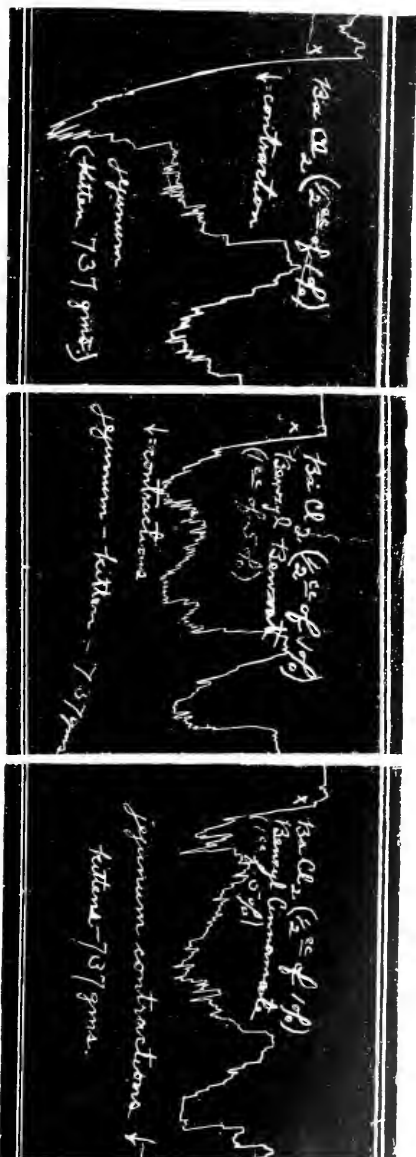


Fig. 4. (For description see text.)

circular intestinal muscles. In our work we have endeavored not to hurry the experiments with barium chloride or by other mechanical means. We have had greater success by awaiting the normal intestinal movements after gently manipulating the organ and setting the apparatus in its proper location for movements of the longitudinal intestinal muscles.

In one of our experiments, however, we did use barium chloride as an intestinal stimulant, but in order to prevent excessive excitability of the organ, we first injected barium chloride alone (1½ c.c. of a 1 per cent solution) and obtained a violent contraction. After allowing the organ to return to normal movements, we now injected the same amount of barium chloride dissolved in the benzyl emulsions. Judging from our tracings (Fig. 4) we feel quite certain that benzyl benzoate as well as benzyl cinnamate diminish the intestinal constrictions produced by barium chloride.

Since benzyl cinnamate appears to be more effective than benzyl benzoate on the intestine, we may here add that it is a solid compound at ordinary temperature but readily melts at body temperature. Similar to benzyl benzoate it is virtually nontoxic when administered orally. We have fed 1½ ounce to a dog weighing approximately ten pounds without any noticeable symptoms.

Another feature we have noted in our experiments is that, after repeated injections of large doses of benzyl esters into the blood stream, the arterial blood becomes very venous in appearance. We have noted no difference in the coagulation period of such blood as compared with normal arterial blood from the same animal.

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## LABORATORY METHODS

### ICE WATER-BATH IN COMPLEMENT FIXATION FOR THE WASSERMANN REACTION—A SHORTENED TECHNIC

BY W. W. DUKE, M.D., KANSAS CITY, MO.

THE refrigeration or ice box method of complement fixation for the Wassermann reaction introduced by Jacobstahl<sup>1</sup> (1910) and Guggenheimer<sup>2</sup> (1911) and popularized by McNeil,<sup>3</sup> Altman,<sup>4</sup> Walker and Swift, Smith and MacNeal,<sup>5</sup> Ruediger<sup>6</sup> and others marks the chief advance in Wassermann technic since the introduction of cholesterinized antigen. The refrigeration method has been used in this laboratory almost exclusively during the past three and one-half years. During this period over fifty thousand tests have been carried out on more than ten thousand different specimens of blood. The ice box method has

been run in parallel with the 37.5° C. incubator method from time to time. Our results harmonize with those reported in the literature showing that the refrigeration method is much more delicate than the warm water incubation method and we believe it will be adopted generally in the course of time.

A serious objection to the refrigeration method has been the time required (4 hours) for incubation. This makes the ice box Wassermann technic require in all about seven hours' time. By using an ice water-bath instead of an ice box, we have been able to shorten the time for incubation to one hour without materially altering its result. We wish, therefore, to report briefly this modification of the method with a few typical examples of reactions which were carried out simultaneously by the warm water-bath, ice box and ice water-bath technic.

#### METHOD

According to the original refrigeration technic, the tubes are set up with antigen, complement and blood to be tested and placed in the ice box at approximately 9° C. for four hours' incubation, after which time the sensitized cells are added and the tubes incubated at 37.5° C. in a water-bath for one hour when the readings are made. By the modified technic to be described, the tubes are set up according to a method to be described subsequently and are placed in a rectangular galvanized iron pan with stopcock for drainage. Ice water is poured into the pan until it reaches a level higher than that of the fluid in the tubes. The temperature of this water is kept at 8° C. for fifteen minutes by the addition of small pieces of ice. (The temperature of a tube placed in the ice water-bath is reduced to 9° C. in five minutes or less.) After the expiration of fifteen minutes, the ice is removed with the result that the temperature of the ice water-bath rises approximately one degree each half hour. At the end of the hour, the ice water is drawn off by opening the stopcock and the pan is refilled with water at 40° C. The tubes are allowed to remain in this for a period of five to ten minutes in order to remove the chill from the tubes before they are placed in the 37.5° C. water-bath. The sensitized cells are then added and the tubes are placed in the 37.5° C. water-bath for one hour at which time the readings are made. The above details are important. If the tubes are not warmed before they are placed in the warm water-bath, the warm water-bath is cooled down with the result that the complement acts too slowly and may make the control tubes simulate the reaction of an anticomplementary serum.

For the past year a five tube test has been used in this laboratory which is set up as follows:

All tubes contain amboceptor (antisheep) two units, patient's blood serum 1-20 c.e., sheep cells 0.2 c.e., of a 5 per cent suspension, antigen 1-5 the anticomplementary amount or less, salt solution to a total volume of 1.25 c.e.

Tube 1	contains,	antigen	cholesterinized	.4%	complement	1 unit.
Tube 2	"	"	"	.2%	"	1 "
Tube 3	"	"	"	.2%	"	2 "
Tube 4	"	"	Acetone insoluble		"	2 "
Tube 5	"	"	Alcohol extract		"	2 "

The advantages of this set up are as follows: Tube 1 is so sensitive that

it comes out positive in the overwhelming majority of specific cases whether active or latent. This tube, therefore, is useful in excluding syphilis in the general diagnosis of a medical case. We find very few cases of syphilis which can be diagnosed clinically or by spinal puncture which do not give a positive reaction in Tube 1. This tube gives a considerable number of false positives, in fact, it gives a 4+ reaction in 33 per cent of the medical cases examined as a routine in this clinic without regard to complaint. (Bloods sent in for Wassermann reactions because of suspected syphilis are excluded from these statistics.) As previously mentioned, this tube is used for the purpose of excluding syphilis in the routine diagnosis of a medical case, not in its diagnosis. Tube 2 is also very sensitive although less so than Tube 1. In fact, it is only about one-fifth as sensitive as Tube 1. However, Tube 2 also gives a number of false positives and gives a 4- reaction in 22 per cent of general medical cases. This test is also used in excluding syphilis, not in its diagnosis. Tube 3, which contains two units of complement, is less sensitive than Tube 1 or 2. It gives fewer false positives. Blood which gives a  $\pm$  reaction in Tube 3 gives a 4+ reaction in Tubes 1 and 2. A 4+ reaction in this tube is not often found in anything other than specific cases. Tubes 1, 2 and 3 come out 4+ in 19 per cent of general medical cases, Tubes 4 and 5, in which two units of complement are used and the antigen is noncholesterinized, are still less sensitive than Tubes 1, 2 and 3. We have no record of these two tubes giving false positives. They come out 4+ in 13 per cent of general medical cases.

It is interesting to compare the reactions of doubtful and weak positive bloods carried out by the three technics, that is, the warm water-bath, the ice box, and the ice water-bath. We have run a great number of parallel tests in this way in the laboratory and find that the ice water-bath and ice box give almost identical results and give invariably a much more complete fixation than the warm water-bath. They are each approximately five times as sensitive as the warm water-bath method. Several typical examples are given in the accompanying tables.

It is interesting to point out in the above tables that the blood used in Table I gave a completely negative test by the warm water-bath technic and gave 4- reactions with cholesterinized antigen and one unit of complement by both refrigeration methods. The blood used in Table II was too weakly positive to inhibit two units of complement by the warm water-bath and gave negative tests in Tubes 3, 4 and 5. It gave 4- reactions in Tube 3 by the refrigeration methods and a 1- and 2- with noncholesterinized antigens. The blood used in Table III was too weakly positive to show any inhibition with noncholesterinized antigens (Tubes 4 and 5) when incubated at 37.5° C. but gave a 4- reaction in these tubes by the refrigeration methods. The above tests are typical examples of reactions given by doubtful or weakly positive bloods.

A clearer conception of the delicacy of the tests can be gained by an examination of Tables IV, V, VI and VII in which several strong positive bloods are titrated and tested by the three methods. In these titrations, cholesterinized antigen, .2 per cent was used, 2 units of complement in Tables IV and V and 1 unit of complement in Tables VI and VII.

TABLE I  
A DOUBTFUL REACTION (TREATED CASE)

TUBE NUMBER	WARM WATER-BATH 1/2 HOUR	ICE BOX 4 HOURS	ICE WATER-BATH 1 HOUR
1	-	4-	4-
2	-	4+	4+
3	-	-	-
4	-	-	-
5	-	-	-

TABLE II  
A WEAK POSITIVE REACTION (TREATED CASE)

TUBE NUMBER	WARM WATER-BATH 1/2 HOUR	ICE BOX 4 HOURS	ICE WATER-BATH 1 HOUR
1	4+	4+	4+
2	4-	4-	4-
3	-	4-	4+
4	-	2+	2+
5	-	1+	2+

TABLE III  
POSITIVE BLOOD (TREATED CASE)

TUBE NUMBER	WARM WATER-BATH 1/2 HOUR	ICE BOX 4 HOURS	ICE WATER-BATH 1 HOUR
1	4-	4+	4+
2	4-	4+	4+
3	4+	4+	4-
4	-	4+	4+
5	-	4+	4+

TABLE IV  
TITRATION OF RELATIVELY STRONG POSITIVE SERUM (2 UNITS COMPLEMENT)

SERUM	WARM WATER-BATH 1/2 HOUR	ICE BOX 4 HOURS	ICE WATER-BATH 1 HOUR
1/36 c.c.	4+	4-	4+
1/60 c.c.	4+	4+	4+
1/100 c.c.	4+	4+	4+
1/200 c.c.	1+	4+	4+
1/500 c.c.	-	4+	4+
1/1000 c.c.	-	4+	3+
1/2000 c.c.	-	-	-

TABLE V  
TITRATION OF STRONG POSITIVE SERUM (2 UNITS COMPLEMENT)

SERUM	WARM WATER-BATH 1/2 HOUR	ICE BOX 4 HOURS	ICE WATER-BATH 1 HOUR
1/30 c.c.	4+	4-	4-
1/60 c.c.	4+	4+	4+
1/100 c.c.	4+	4+	4+
1/200 c.c.	4+	4+	4+
1/500 c.c.	1+	4+	4+
1/1000 c.c.	-	4+	4+
1/2000 c.c.	-	3+	3+
1/3000 c.c.	-	1+	1+
1/5000 c.c.	-	-	-

TABLE VI  
TITRATION OF WEAK POSITIVE SERUM (1 UNIT COMPLEMENT)

SERUM	WARM WATER-BATH 1/2 HOUR	ICE BOX 4 HOURS	ICE WATER-BATH 1 HOUR
1/30 c.c.	4+	4+	4+
1/60 c.c.	4+	4+	4+
1/100 c.c.	4+	4+	4+
1/250 c.c.	1+	4+	4+
1/500 c.c.	-	4+	4+
1/1000 c.c.	-	1+	1+
1/2000 c.c.	-	-	-

TABLE VII  
TITRATION OF STRONG POSITIVE SERUM (1 UNIT COMPLEMENT)

SERUM	WARM WATER-BATH 1/2 HOUR	ICE BOX 4 HOURS	ICE WATER-BATH 1 HOUR
1/30 c.c.	4+	4+	4+
1/60 c.c.	4+	4+	4+
1/100 c.c.	4+	4+	4+
1/250 c.c.	4+	4+	4+
1/500 c.c.	4+	4+	4+
1/1000 c.c.	1+	4+	4+
1/2000 c.c.	-	4+	4+
1/4000 c.c.	-	-	2+
1/5000 c.c.	-	-	1+
1/8000 c.c.	-	-	-

It can be seen in Tables IV to VII that the refrigeration methods (Ice box four hours and ice water-bath one hour) give almost identical results and that both are five times or more as delicate as the warm water-bath method. These are typical examples and represent average results obtained in many comparative titrations. It seems unnecessary to report more examples.

The question which finally arises concerns whether or not one hour's incubation in the ice water-bath gives a finished reaction; in other words, whether or not incubation for a longer period of time or by a different method would give a more complete fixation. To discover if this might be the case, a number of comparative titrations were made in which different methods of incubation were used. It was found that none give a more complete fixation than that obtained by one hour's ice water-bath incubation.

For example, in several series of titrations, the tests were incubated one hour, two hours, four hours and overnight in the ice water-bath. The results obtained were almost identical.

One of this set is reported in Table VIII. It can be seen that with both the noncholesterinized and cholesterinized antigen, the reaction obtained is about the same whether the tests were incubated one, two or four hours respectively. This result was somewhat different from that obtained by incubating bloods for varying periods of time in the warm water-bath. Blood incubated for one-half hour in the warm water-bath does not give as strong a reaction as when incubated for a period of one, two or four hours. In fact, four hours' incubation in the warm water-bath gives a reaction which is definitely stronger than that obtained by one-half hour's incubation. We be-



lieve, therefore, that one-half or one hour's incubation in a warm water-bath is inadequate.

We also made comparative titrations, incubating first in the ice water-bath for one hour and following this incubating in the warm water-bath for one hour; also by incubating one hour in the ice water-bath and then allowing the temperature of the water-bath to rise gradually during a period of nine hours to room temperature and finally incubating one additional hour in the warm water-bath. The results obtained by these methods were little or no more complete or positive than that obtained by one hour's incubation in the ice water-bath. We believe, therefore, that the fixation obtained by one hour's ice water-bath incubation is complete and adequate and is as good as that which can be obtained by any method with which we are acquainted at the present time.

TABLE VIII  
ICE WATER-BATH

ANTIGEN Hrs. Incubation SERUM	2% CHOLESTERINIZED			NON-CHOLESTERINIZED		
	1 HOUR	2 HOURS	4 HOURS	1 HOUR	2 HOURS	4 HOURS
1/30 c.c.	4+	4+	4+	4+	4+	4+
1/60 c.c.	4+	4+	4+	4+	4+	4+
1/100 c.c.	4+	4+	4+	4+	4+	4+
1/130 c.c.	4+	4+	4+	3+	3+	3+
1/160 c.c.	4+	4+	4+	-	-	-
1/200 c.c.	4+	4+	4+	-	-	-
1/260 c.c.	4+	3+	3+	-	-	-
1/340 c.c.	2+	2+	1+	-	-	-
1/500 c.c.	-	-	-	-	-	-
1/750 c.c.	-	-	-	-	-	-

TABLE IX  
WARM WATER-BATH

ANTIGEN Hrs. Incubation SERUM	2% CHOLESTERINIZED				NON-CHOLESTERINIZED			
	$\frac{1}{2}$ HOUR	1 HOUR	2 HOURS	4 HOURS	$\frac{1}{2}$ HOUR	1 HOUR	2 HOURS	4 HOURS
1/30 c.c.	4+	4+	4+	4+	4+	4+	4+	4+
1/60 c.c.	4+	4+	4+	4+	4+	4+	4+	4+
1/100 c.c.	4+	4+	4+	4+	3+	4+	4+	4+
1/130 c.c.	4+	4+	4+	4+	2+	1+	3+	3+
1/160 c.c.	2+	3+	4+	4+	-	-	1+	-
1/200 c.c.	1+	2+	3+	4+	-	-	-	-
1/260 c.c.	-	1+	3+	3+	-	-	-	-
1/340 c.c.	-	-	2+	2+	-	-	-	-
1/500 c.c.	-	-	-	-	-	-	-	-
1/750 c.c.	-	-	-	-	-	-	-	-

#### CONCLUSIONS

1. The use of an *ice water-bath* for *one hour* for complement fixation for the Wassermann test gives as complete a degree of complement fixation as incubation in the *ice box* for *four hours*. The use of an ice water-bath, therefore, shortens the refrigeration technic three hours without altering its accuracy. For this reason its use is recommended.

2. I acknowledge with pleasure the expert assistance in this work of Miss Elizabeth Leas, technical laboratory assistant.

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## HEMOSTATIC AGENTS\*

## A FURTHER NOTE ON THE DETAILS IN THE PROCESS OF ASSAYING

BY HERBERT C. HAMILTON, DETROIT, MICH.

IN an article on "Hemostatic Agents"<sup>1</sup> (this JOURNAL, June, 1920) I described a method by which one class of these agents can be standardized in spite of the fact that the action is not directly that of a coagulant, but, on the contrary, is of such a character as slowly to change the coagulability of the blood when it is administered either internally or hypodermatically. Certain features of this method have been called in question by Hanzlik<sup>2</sup> (this JOURNAL, November, 1920) and since the criticisms concern not only the good faith and intelligence of the author, but the scientific accuracy of the method in several of its details, I feel justified in restating some of the principles of the test and the reasons for the deductions and conclusions which followed.

The method is in brief an adaptation of the clinical method of using this particular class of hemostatics. The dog is anesthetized for convenience of manipulation and the coagulation time of the blood carefully observed before and after administering the agent. The amount of blood drawn from the circulation is approximately 3 to 5 c.c. The end point for any sample of blood is complete coagulation so that the tube can be inverted and shaken with no evidence of fluid blood.

In spite of the apparent simplicity of the method certain conditions have been specified as prerequisite to a careful scientific demonstration of the value of the agent. These are as follows:

Animals are to be rejected for test purposes—first, if the normal coagulation time is less than six minutes; second, if the coagulation times of the samples of blood drawn to establish the normal, are found to vary greatly, particularly when the time becomes shorter; third, if a dog fails to react to a sample which had been demonstrated to be an active coagulant by tests on two other animals.

Records of experiments on 70 dogs show the average normal coagulation time to be eight minutes. This series includes five dogs whose normal coagulation time was found to be five minutes or less and three with coagulation time of twelve minutes or more.

\*From the Research Laboratory of Parke, Davis and Co., Detroit, Mich.

The average time being eight minutes, it is logical to exclude animals with a time materially less. A blood coagulant is intended primarily for cases not exceptionally short but either normal or exceptionally long. Since the selected standard for Hemostatic Serum is that it must shorten the time to one-third the normal and since the usual shortening is considerably greater than this, five to six minutes is regarded as the shortest normal time that could be permitted as being consistent with a test designed to recognize the highest efficiency of the agent.

With regard to the second reason for rejecting a dog—the abnormal variability of its coagulation time—the reason is evident. It is impossible to select a normal on which to base the action of the agent. If the time varies from nine minutes to three-fourth minutes as observed by Hanzlik in a previous experiment, it is not possible to select a normal time to be shortened to one-third by the agent.

With regard to the third cause for rejecting a test animal, namely, failure to respond to a sample of known activity, it may be said that pharmacologists are obliged to accept results from a majority of animals used: not every animal is adapted to test purposes. Two out of three, three out of five or five out of seven are commonly accepted as sufficiently accurate results in all biologic assaying. Reference to almost any data of this character will show the correctness of this statement.

Another point to which Hanzlik took exception is the precaution to assure oneself as to the character of the clot. While we agree as to the end point, i. e., invertibility of the tube without a trace of fluid blood, there are occasions when this condition is apparent and not real and fluid blood is present beneath the superficial skin. Which is better, to be misled in thinking a clot has formed when it has not, or to assure oneself by carefully breaking the surface to observe the condition of the major portion of the sample? The answer seems to me to be obvious. It is like one of Euclid's axioms. A blood clot is a true clot when none of the blood remains fluid.

The manipulations used to ascertain the character of the clot are indeed not conducive to the best interest of the clotting process. According to the most elementary rules of logic, these adversely disturbing factors should be held as corroborative of the value of the test. This author's position in criticising what is evidently a meritorious precision in reading the tests is to say the least beyond comprehension.

Another point was noted in the original communication that several observers have found hemorrhage to be self-limiting. While many observations of my own seem to discredit this statement, the possibility that repeated withdrawals of blood might vitiate the test has not been disregarded. The observations of Drinker and Drinker,<sup>3</sup> *Am. Jour. Phys.*, 1915, xxxvi, were to the effect that "Rapid progressive hemorrhage causes a progressive decrease in coagulation time." The data on which they based the statement is a record of experiments on rabbits and cats in which the maximum shortening was 37 per cent. The detailed experiments included one in which 60 c.c. blood was withdrawn from the vein of a 2 kilo cat. The original coagulation time of 14 minutes was

shortened to 12 minutes, an actual shortening of about 15 per cent. While the authors did not make the calculation, it is well known that the average blood volume of the cat does not exceed one-ninth the weight of the animal. It is a fair inference, therefore, that the amount of blood drawn is approximately 33 per cent of the total volume in this test animal.

In the other detailed experiment of Drinker and Drinker 50.5 c.c. blood was drawn from a 2.6 K. rabbit. The shortening of coagulation time was in this case from 18 minutes, 32 seconds to 15 minutes, 33 seconds, a total of 3 min. or about 17 per cent. By the same process of calculation, the amount drawn was about 20 per cent of the total volume.

Note: The quantity of blood in the average animal is approximately one-thirteenth of the body weight<sup>4</sup> (Luciani's Human Physiology, Vol. I, p. 99). This varies so greatly, however, that one-ninth was selected as being the highest possible ratio.

The large proportion of the blood drawn and the small decrease in coagulation time in these experiments indicate that hemorrhage is not a serious factor as influencing coagulation time.

Gray and Lunt<sup>5</sup> (Am. Jour. of Phys. Vol. 34) conclude that "Hemorrhage decreases clotting time especially if moderately severe—13 per cent of the circulating blood."

Hanzlik quotes Howell<sup>6</sup> (Harvey Lectures (1916-17) p. 296) to the effect that "results of these experiments [cephalin on dogs] have not been published since they showed *many irregularities* [italics Hanzlik's] that will require further experimentation to explain." Without the details one is at liberty to assume that it is the action of the cephalin rather than any other factor that showed "many irregularities." Howell<sup>7</sup> (loc. cit.) recorded one experiment where cephalin was injected intravenously in which the coagulation time was shortened by one-third to one-half of the normal. He did not hesitate to credit this change to the cephalin. The technique of the experiment was not given in detail but there is no evidence that he feared the influence of hemorrhage.

Cannon and Gray<sup>8</sup> (Am. Jour. of Phys. Vol. 34) used a similar method to prove that adrenalin shortens the coagulation time. They prove by seven or more experiments on cats that the time can be shortened over a period of about 20 minutes by injection of adrenalin, after which the coagulation time returns to normal. In several experiments they show no material change in coagulation time when no adrenalin was injected thereby demonstrating the correctness of their deductions and the accuracy of the method.

In view of the additional references in which a method similar to the one I described was applied and further in view of the fact that hundreds of physicians report the satisfactory use of hemostatic agents which Hanzlik tested without finding them effective, it is fair to conclude that his methods or his technique were at fault.

The remarkable properties of Hemostatic Serum (Hemoplastin) by virtue of which it shortens the coagulation time of the blood was first demonstrated clinically. It was not until several years afterward that this method was selected as a means of standardization. Other methods fail to show its effective-

ness because of the character of this agent. Its action is to change the character of the blood so that the coagulation time is shorter and not, as is the case with most hemostatics, a local action on the escaping blood only.

Progress in scientific achievements is marked by improved products and improved methods. In the case of a new or improved product, the improvement being susceptible of clinical demonstration, it is not illogical to expect that a change in method may be necessary to demonstrate that value in the laboratory. In the present instance the laboratory method selected for demonstrating the activity of the product is almost identical with the clinical use of the agent. When it is demonstrated that the clinician has been misled as to its value, then, and not till then, will it be logical to conclude "that Hemostatic Serum (Hemoplastin) is inert \* \* \* as a hemostatic *in vivo*."

Hanzlik's more detailed description of his method of obtaining samples of blood throws considerable light on the peculiar results obtained. He says\* (this Journal Nov. 1920, page 59) "A small seraffine clip was placed on the vessel central to the cannula. When the hemorrhages were made, the clip was loosened and 1 to 2 c.c. of blood allowed to escape into small vials of 4.5 cm. length and 1.2 cm. in diameter and with straight sides. Duplicate tests were made requiring the withdrawal of about 4 c.c. of blood at most into the vials plus the small quantity of blood left behind in the cannula \* \* \*. In several instances it was possible to invert the vials without loss of contents almost immediately after completion of the hemorrhage or in about 10 seconds."

In his original communication he records a change from 9 min. for complete coagulation to  $\frac{3}{4}$  min. and one may assume that only 8 c.c. of blood was drawn. Is it probable that this small loss of blood would occasion so great a change in the rate of coagulation?

An unbiased observer would say that this change in coagulation time is due not to any change in the circulating blood but only in the escaped blood and further that this change is because the blood remaining in the cannula has liberated some of its thrombin and thrombokinase to the blood being drawn into the test sample thereby accelerating the clotting process. In addition to this is the effect of the seraffine clip which would undoubtedly compress the artery sufficiently to squeeze out some plastic lymph containing thrombokinase which adds still further to the acceleration in clotting. These details which as Hanzlik said "were omitted from our original paper, because we considered them sufficiently implied in the data given" are quite necessary to be known for otherwise one would assume that reasonable care had been taken to eliminate such obvious errors in technique. And indeed the results obtained by the author are entirely due to these technical errors.

Care must be taken not to traumatize vessel used in obtaining the blood. Cannula or needle used in withdrawing blood must be perfectly clean and must be cleaned at each bleeding with saline solution. It is obvious that the condition of the cannula is of paramount importance in the tests since if allowed to remain *in situ* the film of blood adhering to its walls would liberate sufficient Kinase to accelerate the clotting processes at subsequent bleedings. In order to carry out the test in such a way as to minimize still further the effect of faults in

technic, I have carried out tests on horses the protocols of which follow. The method used was to insert a trocar with its cannula into the vein and obtain the blood by withdrawing the trocar allowing a quantity of blood to escape and be rejected before saving the sample for observations.

The weights of the horses used were approximately 500-600 kilos. The samples of blood varied in quantity but were approximately 20 c.c. The temperature of the water-bath into which the tubes were placed was 38° C. Observations were made at 1 minute intervals, the records being made by symbols,—1, 2, 3, 4, which indicate the extent of coagulation as it progresses from (-) when no fibrin shows to (4) which is a complete clot with no liquid blood in evidence. With this description of the technic the protocols seem to need no further explanation.

No additional data on dogs—the regular test—are included at this time since the two previous articles<sup>19</sup> (this JOURNAL, June, 1920) and J. A. Ph. A. Feb. 1920) contain results typical of those obtained in testing 35 lots of Hemostatic Serum (hemoplastin).

It should be noted here that the amounts injected into the horses were varied in order to find what dose is necessary to bring about a reasonable degree of shortening in the coagulation times of these animals, no previous work having been done on horses. While an insufficient amount of experimentation has been done to establish a dosage it is evident that 25 to 30 c.c. might be regarded tentatively as an average dose.

The clinical results now extending over three years of time, the coagulation studies in men, (made not only by test tube methods, but also by the Biffi-Brooks coagulometer in which instance only four drops of blood are used at each study), the physiological testing on animals of thirty-five (35) different lots, and the studies made on horses and cows, the protocols of which are here appended, all uniformly show the activity of Hemostatic Serum in reducing the blood clotting time. We feel warranted in concluding that the activity of Hemostatic Serum in reducing the coagulation time of blood can now be regarded as a matter belonging to the domain of established facts.

HORSE No. 525											
	1	2	3	4	5	6	7	8	9	10	minutes
1:52	—	—	—	1	1	1	2	2	3	4	
1:57	—	—	1	1	2	3	3	4			
2:05	—	—	1	2	2	3	3	4			
2:16	—	—	1	2	3	3	3	4			
2:18	Injected 25 c.c. Hemoplastin 054981										
2:52	1	2	4								
2:58	2	3	4								
3:02	2	4									
3:05	1	1	3	4							
3:11	1	3	4								
3:18	1	1	2	4							

Horse quite noticeably affected as shown by labored respiration and lassitude.

## HORSE No. 90. NO INJECTION

	1	2	3	4	5	6	7	8	9	10	11	minutes
3:26	-	1	1	2	2	2	3	3	4			
3:45	-	-	1	2	3	3	3	4				
3:50	-	-	1	2	2	3	3	4				
3:57	-	1	1	3	4							
4:04	-	-	-	1	1	2	2	3	4			
4:12	-	-	1	1	2	3	3	4				
4:18	-	-	-	1	1	2	3	4				
4:23	-	-	1	2	2	2	3	4				
4:31	-	-	1	2	2	3	3	3	4			
4:38	-	-	1	1	1	2	2	3	4			
4:42	-	-	1	2	2	3	3	4				

No injection and no marked change in coagulation except in one instance.

## HORSE No. 64

	1	2	3	4	5	6	7	8	9	10	11	minutes
9:38	-	-	1	1	2	2	3	3	4			
9:42	-	-	1	1	2	3	3	4				
9:47	-	-	1	1	2	3	3	4				
9:52	Injected 10 c.c. Homoplastin 054981											
10:39	-	-	1	2	2	3	3	4				
10:51	-	-	1	3	4							
10:56	1	2	3	4								
11:04	1	3	4									
11:08	1	2	3	4								
11:14	1	2	3	3	4							

There was no evident reaction other than the shortening of coagulation time from 8 min. to 3 to 4 minutes.

## HORSE No. 1129

	1	2	3	4	5	6	7	8	9	10	11	minutes
11:22	-	-	1	1	2	3	3	4				
11:29	-	-	1	2	2	3	3	4				
11:31	-	-	1	2	3	3	4					
12:00	Injected 15 c.c.											
1:21	-	1	1	2	3	3	4					
1:27	-	1	2	2	3	3	3	4				
1:34	-	1	2	3	3	4						
1:42	-	1	2	2	3	4						
1:49	-	1	2	3	4							

No evidence of a reaction except the shortened coagulation time.

## HORSE No. 72

	1	2	3	4	5	6	7	8	9	10	minutes
2:23	-	-	1	1	2	3	3	4			
2:28	-	1	1	2	2	3	4				
2:34	-	1	1	2	3	3	4				
2:39	Injected 15 c.c. 054981										
3:10	-	1	1	2	4						
3:15	-	1	2	3	4						
3:19	1	1	2	3	4						
3:23	-	1	2	3	4						
3:30	-	1	3	3	4						

## HORSE No. 72

	1	2	3	4	5	6	7	8	9	10	minutes
3:38	1	2	3	4							
3:42	1	2	3	3	4						
3:48	1	2	3	4							
4:00	1	1	2	3	4						

No evident reaction except a slightly shortened coagulation time.

## TRINX 67

	1	2	3	4	5	6	7	8	9	10	11	minutes
2:38	—	—	1	2	3	3	3	4				
2:43	—	—	1	1	2	3	3	4				
2:48	—	—	1	2	2	3	3	4				
2:52	Injected 30 c.c. 54981											
3:30	—	1	2	3	3	3	4					
3:37	—	1	1	2	3	3	3	4				
3:45	—	1	1	2	3	4						
3:51	—	1	2	3	4							
3:55	1	1	2	3	4							
3:58	1	1	3	3	4							
4:04	1	2	3	4								
4:10	1	1	3	4								
4:14	1	3	4									
4:21	1	3	4									
4:25	2	4										

No evidence of a reaction other than the shortened coagulation time.

## TEST OF HEMOPLASTIN ON A HEIFER

	1	2	3	4	5	6	7	8	9	10	11	minutes
10:34	—	—	1	1	2	2	3	3	4			
10:35	—	—	—	1	1	1	2	2	3	3	4	
10:36	—	—	—	1	1	2	2	2	3	3	4	
10:40	Injected about 15 c.c. Rx 054981											
11:16	—	1	2	3	4							
11:22	—	1	2	3	3	3	4*					
11:28	1	2	4									
11:31	1	3	4									
11:36	1	2	3	4								
11:41	1	2	3	4								
11:45	1	2	3	3	4							

\*Clotting interfered with at 4 min. This influenced the coagulation to such an extent that the reading is incorrect. Actual time 4 to 5 minutes.

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- <sup>4</sup>Human Physiology, i, p. 99.
- <sup>5</sup>Am. Jour. Physiol., xxxiv, p. 351.
- <sup>6</sup>Harvey Lectures, 1916-17, p. 296.
- <sup>7</sup>Loc. cit.
- <sup>8</sup>Am. Jour. Physiol., xxxiv, pp. 232-242.
- <sup>9</sup>Jour. Lab. and Clin. Med., November, 1920, p. 59.
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## EDITORIALS

### *Biochemical Changes in Traumatic Shock*

PREVIOUS to the World War it was generally held from experimental work that shock was due to deficient blood or fluid contents of the blood actually circulating in the vessels. The theory of splanchnic congestion held large sway. A most important study on the causation and pathology of shock was made by a special investigation committee, appointed by the British Medical Research Committee. Through their work we have acquired a much more complete picture of the process actually occurring. Attention has recently been directed not so much the physiologic changes as to biochemical reactions occurring in shock. As a result of investigations along these lines we can now hypothecate quite clearly the various changes occurring during shock.

During the war shock followed trauma and was apparently increased by hemorrhage, pain, exposure to cold, mental distress and rough handling of the wounded. It was soon recognized that the low blood pressure could not be explained by a collection and stasis of the blood in the veins of the abdominal viscera. This was amply demonstrated in large series of abdominal operations.

Wallace in his report emphasizes the importance of differentiating between primary shock which may be analogous to faint or collapse and which usually comes on immediately after the receipt of an injury, and secondary wound shock which usually does not occur until after the exciting causes above enumerated have had time to get in their effect. He suggests that primary shock may be the result of afferent impulses exhausting the vasomotor system, but that the information recently acquired concerning secondary shock indicates a toxic origin. The fact of its late development corresponds with the time required for the elaboration of the toxin.

The most important phenomena observed early in the war and forming the basis of the new studies, were, first the fact that congestion of the splanchnic vessels did not occur; second, that there was no loss of fluid into the tissues sufficient to cause edema; third, that as shown by the vital red method a shocked man had a reduced blood volume, and fourth that the power of recovery was dependent to a great extent upon the power of circulation to take up fluid from the tissues or to retain added fluid. Those cases of shock which still retained the ability to keep the blood diluted did well under treatment, while those in which this power was feeble or lacking, did not respond to therapeutic measures.

Wallace points out that shock is usually the result of several factors such as hemorrhage, toxins, cold and anesthetics. It usually does not result from any one of these factors acting alone. A considerable loss of blood may be compensated by the drawing of fluid from the tissues into the vessels; or toxic products alone may be destroyed or excreted. But a combination of these two processes makes the work more difficult. Sometimes recovery follows the combined action of even three factors, but if in addition operation is required and an anesthetic with its toxic features is superadded, the result may be fatal.

The work of Crile and others has suggested that shock is due to failure of the vasomotor mechanism. But Malcolm, Henderson and Mann and others have shown that the vasomotor center is active during shock and that the defective filling of the heart is due rather to a reduction in the volume of circulating blood. This reduction appears to be due to a loss of plasma from the blood. Such a loss has been explained in shock following etherization by the fact that toxic damage to the capillary endothelium renders it abnormally permeable.

Dale, Laidlaw and Richards, have produced shock experimentally by the intravenous injection of histamine. Histamine when administered parenterally produces symptoms similar to those following injection of the protein poison described by Vaughan and as stated by Vaughan is probably quite similar in its chemical constitution.

When injected in small amounts the drug produces a lowering of the blood pressure, apparently due to simple vasodilatation. In larger amounts it causes in the experimental animal a typical shock-like failure of the circulation, with arterial constriction, oligemia, concentration of the blood, and failure of cardiac output. In animals, shocked with histamine the blood does not accumulate in the large arteries or large veins, nor in the liver or spleen.

There develops a much higher concentration of red blood cells in the blood than before the advent of shock. This change is due apparently to the passage of some plasma out of the vessels into the tissues. The loss of plasma in some cases amounts to as high as 50 or 60 per cent of the original plasma volume, or 40 per cent of the entire blood volume. In addition blood accumulates in the venules of the intestines and presumably also in those of the skeletal muscles and elsewhere. The arteries however, even down to the smallest arterioles remain consistently constricted. Dale and his associates believe that the most important early phenomenon occurring in shock is the tendency of the blood to accumulate in the capillaries and that the essential cause of the condition is a general loss of the normal tonus of the capillary walls. It is a fact that normally only a small portion of the capillary net work of the body is functioning at any given time. Many capillaries are entirely collapsed. Following a call for increased blood volume to any particular tissue the capillaries in that tissue dilate, thereby producing a local increase in blood flow. The authors suggest that under the influence of a large dose of histamine the capillary tone is lost throughout the body, the whole of the potentially available capillary bed becomes simultaneously patent, and the blood percolates into the network of channels as water into a sponge. In this type of shock the rapid diminution of outflow from the organs into the veins causes a fall in venous pressure. The heart is then not filled in diastole and its output rapidly declines. The arterial pressure declines but this is delayed slightly by arterial constriction in the peripheral vessels. In animals shocked with large doses of histamine there is added to this the passage of plasma through the vessel walls into the tissues following damage to the capillary endothelium.

The investigators point out that the phenomena of local inflammation are of the same nature as the general changes seen in shock; abnormal permeability of capillary walls, transudation of plasma, concentration of the blood by loss of plasma and stagnation in dilated capillary channels.

Dale, Laidlaw and Richards point out that shock usually follows extensive tissue damage, as laceration of muscles, liver or intestines and that decomposition products are known to be formed in the body-tissues having an action of the so-called "histamine type."

Dale in studying the results of the combined action of histamine and anesthetics, found that while relatively large doses of the poison alone can be tolerated by the experimental animal, much smaller doses cause fatal shock in animals which have previously undergone a prolonged ether anesthetization. A conspicuous feature of the histamine shock in an anesthetized cat is the rapid concentration of the blood by loss of plasma and the associated stasis in the capillaries. He assumes that one factor in the reduced tolerance to histamine produced by ether is a weakened resistance of the capillary endothelium. This weakening once produced persists for some time after removal of the anesthetic. The loss of plasma is less conspicuous following hemorrhage than following ether administration.

Dale, Laidlaw and Richards, while hypotheacating the very interesting theory of histamine shock, admit that they have been unable with the technique at their disposal to demonstrate a local dilatation of the capillaries as a

result of histamine application. This should be the first step in the testing out of their hypothesis. Rich, working in the pathologic laboratory of Johns Hopkins Medical School has supplied the missing evidence. After perfecting an ingenious cabinet in which to examine experimental animals, he examined the omentum, left in the abdomen but placed on the stage of a specially constructed microscope. He observed the capillaries both before and after the local application of histamine di-hydrochloride and found no change in the size of the capillaries or in the rate of flow through them. Nor did he find that any additional capillaries opened up which had not been visible before the application of the drug. He next observed the omentum in the same manner before and after the production of shock by intravenous injection. Here he demonstrated a pronounced slowing of the capillary stream accompanying the fall of blood pressure, but no widening of the capillary bed. Next he applied strong irritants locally and in the same manner as he had applied the histamine. He again obtained negative results.

Rich later proved that even the moderate manipulation required to place the omentum on the stage of the microscope was sufficient to produce dilatation of the capillaries, so that even in his controls the condition for which he was searching had already occurred. This was accomplished by opening the abdomen and flooding it with a fixing solution, removing the omentum and examining it after staining, under the microscope. This process was carried out before and after manipulation of the omentum and again after the local application of histamine. He concluded from these studies that handling of the omentum opens the normally collapsed capillaries, either by causing a loss of tone or by stimulating them to dilate. Local application of normal salt solution did not affect the capillaries in any way, but histamine applied locally produced markedly dilated tortuous capillaries engorged with blood. There was also an absolute increase in the number of visible capillaries. The dilation was not confined to the capillaries but included the smallest arterioles and venules at the periphery of the capillary bed. That the local application of the poison gives only a local reaction is shown by the taking of blood pressure tracings during the experiment. The pressure was maintained at a constant normal level.

Rich next examined the fixed tissues of the omentum, after producing histamine shock by intravenous injection. The capillaries and smaller arterioles and venules of the shocked animal were definitely dilated and engorged with blood, while many occult capillaries had been opened up. In this way he demonstrated that a widespread peripheral vascular dilatation occurred of such a degree as to seriously impair the circulation. Control animals did not react in this manner.

In the experimental shock thus produced there was a fall in blood pressure corresponding to the dilation of the peripheral capillaries. The dilatation began within 15 seconds after injection and there was no recovery from this dilatation even at the time of the secondary rise in blood pressure. This secondary rise has been ascribed by Dale and Laidlaw to a constriction of the pulmonary arteries. Rich also confirmed the observations of Dale and Laidlaw regarding the excellent functional condition of the heart in histamine

shock. Death during shock was always due to respiratory rather than cardiac failure. The inadequate venous return to the heart was also observed.

Thus Rich has confirmed the work of the other investigators and has carried our knowledge one step further.

The amino-acid histidine is one component of practically every body protein. Histamine is derived from this amino-acid by decarboxylation. Comparison of histamine with Vaughan's protein poison shows striking similarities. The latter also is present in all body proteins. Typical anaphylactic shock can be produced by either substance. The symptoms and pathologic findings are practically identical. Vaughan writes, "With our poison histamine seems to agree closely. Both induce bronchial spasm and distension of the lungs in guinea pigs and cause prompt and marked fall in blood pressure in dogs. Neither destroys the coagulability of the blood. In the purest form in which we have obtained it, our poison kills guinea pigs intravenously in doses of 0.5 mg. and this is the fatal dose for histamine. When the active agents in our crude poisons are isolated we shall not be surprised if histamine or some closely allied body is among them."

Bayliss and others have shown that acidosis is not the cause of traumatic shock. The intravenous injection of solutions of sodium bicarbonate have only a brief effect in raising the blood pressure and if small doses are given during the development of shock, they have no power to prevent its appearance. The injection of lactic acid during the state of shock from muscle injury does not in any way exaggerate the condition and in some cases has been found to benefit it by raising the blood pressure. Lactic acid is therefore not the causative agent. Nor are other acids as such the causative agents. Bayliss concludes that some other substance produced by tissue disintegration must be responsible and he turns to histamine or some related compound to explain the condition.

He found through experimental investigation that the blood pressure can be permanently restored by gum acacia solution injected intravenously. Also the development of shock from even very severe muscle injury could be prevented by repeated small injections of gum, as the blood pressure fell from time to time. He found it quite effective as late as five and one-half hours after the injury provided the blood pressure had not been so low as to cause paralysis of the nerve centers. Bayliss concludes that the toxic products can be removed or destroyed as quickly as they pass into the blood current if the volume of blood is kept sufficiently large to ensure a good circulation and supply of oxygen to the tissues. Whether the toxic products are oxidized as is lactic acid or whether they are rendered harmless by passing through the liver is unknown. He points out that histamine disappears very rapidly from the circulation if the circulation is effective. He further states that in shocked soldiers in which there was no evidence of much hemorrhage there was permanent improvement following the intravenous injection of gum solution.

Cannon and Bayliss have shown that extensive muscle injury in the experimental animal will result in a fall of blood pressure and the symptoms

of shock. Also that massage of the damaged tissue increases the condition of shock. They point to the analogy in the case of traumatic shock in men.

Cannon showed that traumatic experimental shock was not due to nervous stimulation, by producing the condition in animals by damage to the muscles after preliminary severance of the cord in the lumbar region. He produced shock in cases in which the nerves to the limb traumatized had previously been severed at the point of their emergence from the pelvis. Finally he showed that shock was not produced even though the nerves were intact, when the blood vessels leading from the damaged limb were previously ligated. If the blood vessels were subsequently opened and the blood was permitted to flow in and out of the damaged region as long as 33 minutes after the trauma, shock appeared. Lastly, if after shock had been established the blood vessels to the injured leg were ligated the symptoms of shock soon commenced to disappear.

It is of considerable interest to compare the preceding observations with those made by McNee and his associates in the treatment of wound shock in soldiers. The phenomena which they attempted to combat were first the lowering of the body temperature, second the lowering of the blood pressure, and third, the diminution of the blood volume. The two latter were treated by either transfusion of blood or by the injection of gum salt solution intravenously. The dramatic effects with blood transfusion were not seen following infusion with gum salt. The difference is however superficial, due to the fact that with blood the man loses his ashen hue, the face and lips redden and the man's general appearance alters completely. With gum solution, the red corpuscles being absent, there is no such objective change and the observer must look deeper than the skin for evidences of improvement. Above all he must watch the blood pressure. It was the case of hemorrhage combined with not more than a very moderate degree of shock which constituted the successful cases. Those cases with severe shock and little hemorrhage did not respond nearly so readily to treatment. Administration of sodium bicarbonate intravenously never resulted in any apparent benefit. An observation of striking significance was the fact that in those cases severely shocked there was usually very extensive injury to muscle tissue. When the wound consisted of a severe laceration of a limb it was sometimes possible at the height of the improvement to perform a very rapid "guillotine" amputation. This operation was commonly followed by a remarkable and maintained improvement. Occasionally the application of a tight tourniquet above the site of the muscle injury produced similar good effects.

Another correlation between the experimental findings and the observations in wounded men was made by Keith, who studied blood volume in wound shock. He found that in individuals who had undoubtedly lost a considerable amount of blood the red corpuscles and hemoglobin estimation frequently closely approximated the normal. In cases of extremely severe primary hemorrhage, the reduction in corpuscles and hemoglobin percentage was strikingly small in view of the actual amount of blood lost. The red cell content per volume of blood appears therefore to be of less importance in shock than is the total amount of the circulating blood. Studying this latter factor Keith found that the *total* blood and plasma volume were consistently reduced, thus

explaining the anemic condition of the patient in spite of a relatively high blood count. He also showed that the lessened blood volume bore a definite relation to the severity of the clinical symptoms. In a patient with distinct symptoms of shock the blood volume ranged from 51 to 85 per cent of normal and there was a corresponding reduction of plasma.

That this deficiency of blood was not due entirely to loss of blood from hemorrhage but that other factors played an important part is indicated by the fact that donors for transfusion who frequently lose as high as 800 c.c. of blood do not suffer from the same symptoms. In fact Keith found that in these latter the blood volume frequently returned to normal within one hour's time. Even without hemorrhage shock may be accompanied by a fall of blood volume. This is not accompanied by a fall in hemoglobin, but the hemoglobin percentage rises. Thus a rising hemoglobin in shock indicates a bad prognosis. The loss of plasma results in a concentration of red cells and of hemoglobin. There are other factors in the production of the low blood volume observed in wound shock than the actual amount of blood lost.

Keith studied the reaction during the injection of six per cent gum acacia solution, a mixture having the same viscosity as whole blood and the same osmotic pressure as plasma. This solution frequently gave results entirely comparable to those following transfusion. In cases recovering after injection of gum solution there was always demonstrated an actual increase in plasma and in total blood bulk. In those cases that did not respond to vigorous treatment with gum solution, the trouble appears to have been due to an inability of the vascular system to retain the added fluid. Thus in one case, one hour after 1000 c.c. of gum solution had been injected the increase in total blood volume was only 200 c.c. Autopsy in those cases failing to react frequently showed markedly edematous lungs and subcutaneous tissues.

White and Erlanger have experimented with the intravenous injection of gum solution and glucose in shocked animals. The solution used consisted of 18 per cent glucose and 25 per cent gum acacia. They found that in shock the concentration of the protein in the plasma was not appreciably changed and assumed therefrom that in shock the permeability of the vessel walls was increased. After injection of the solution the blood volume increased from the shock level and continued to increase to even above the initial normal level. After this it again fell off gradually until at the end of seven hours it reached approximately its initial level.

Regarding the plasma protein it had been found as stated that the concentration under shock was practically unchanged from the normal concentration, but that the absolute amount of plasma protein was greatly diminished to correspond with the diminution in blood volume. With the increase of blood volume following injection the absolute amount of protein increases definitely but not as rapidly as does the volume. In other words the fluid drawn in is not so rich in protein as is the plasma but neither is it protein free. When the blood volume subsequently begins to fall away the protein continues to increase even while water is passing back out of the vessels. The protein concentration rises faster than the plasma volume falls. White and

Erlanger suggest as an explanation that when the injection is being given the fluid drawn in brings with it protein through the abnormally permeable walls but in lowered concentration than it occurs in plasma. Then as the blood pressure rises the circulation improves and lymph flow is reestablished. The lymph flow from the liver and intestines is probably accelerated to a greater degree than from the extremities. As the circulation to these organs is improved, the normal lymph flow is reestablished and the lymph carries back into the blood stream through the thoracic duct the plasma protein which has accumulated in the tissue spaces of the liver and intestines, during the induction of shock. Thus protein is entering the blood stream even while the blood volume is falling.

The work of White and Erlanger on normal and shocked animals indicates that the hypertonic glucose solution first draws fluid into the vessels and that as the sugar rapidly disappears from the blood the fluid is retained in the circulation by the gum acacia solution.

Aub has recently studied the basal metabolism in shocked animals. He finds that experimental traumatic shock causes a marked fall in the rate of basal metabolism to 70 per cent of the original level. The degree of fall is dependent upon the severity of the shock produced. Shocked individuals not only lose heat rapidly by sweating but they also fail to produce sufficient heat to keep themselves warm. Recovery from shock after blood transfusion is usually associated with a prompt return of the metabolic rate to a normal level. As an explanation for this fall in metabolic rate Aub and Cunningham find a markedly diminished oxygen content of the venous blood. This change occurs before the blood pressure falls to a shock level and is still present after apparent recovery from shock. The blood flow is also greatly decreased in the development of, during and after shock. They suggest that the anoxemia of the tissues may be the cause of the decreased metabolism. The decreased blood flow and the reduced oxygen content of the venous blood results in a secondary fall in metabolism. The blood flow becomes markedly slowed before the onset of a shock level of blood pressure and the metabolism does not fall until later.

They point out that a vicious circle is formed. As an oxygen want develops in contracting muscles, many empty capillaries fill with blood. This stage reduces the distance necessary for the diffusion of gases into the tissues. Thus as anoxemia develops the capillary bed increases in volume. This further decreases the already slow blood flow and the slower the flow the greater becomes the oxygen consumption per cubic centimeter of blood. This produces a decrease in oxygen content of the venous blood.

Aub and Wu in investigating the chemical changes in the blood during shock, found that animals with marked muscle trauma but without true shock, showed only slight changes in total nonprotein nitrogen, urea, creatin and sugar in the blood. All of these constituents increase as shock develops but this was especially true of the creatin and the sugar. The marked rise in creatin is direct evidence of the presence in the blood of products of muscle necrosis and therefore suggests that the theory of the chemical causation of traumatic shock is probably the correct one. The rise in blood during shock is not entirely explained.



Keith outlines a method to be used in combating traumatic or surgical shock, based upon the foregoing theory of causation. He divides clinical cases into three groups. First the "compensated" cases. In this type the patient has no special symptoms except pain at the location of the wound and feeling of general weakness. The pulse is increased to 90 or 110. The systolic blood pressure remains above 110 and the blood volume is never reduced below 80 per cent. The treatment in this type consists of rest in a warm bed and if symptoms of failing compensation appear during or after operation, the administration of gum solution in 500 c.c. amounts. Saline by rectum may be given either by continuous drip or by injections of 400 c.c. every two hours.

The second group designated "partially decompensated," consists of those in which the pulse rate is between 120 and 140 and difficult to count, the systolic blood pressure is below 90, usually 70 to 80, and the blood volume ranges between 65 and 76 per cent. The patient is usually very pale, restless, thirsty and vomits readily. The extremities are cold and partially anesthetic, to painful stimuli. The management is more difficult. The application of heat is important. If after one or two hours the pulse is not improved, fluid should be administered either by rectum or by gum solution, 500 c.c. amounts intravenously. This should be checked by hemoglobin estimations. A rising hemoglobin after intravenous administration is of bad prognostic significance. The pulse and blood pressure readings are reported at half hourly intervals following the infusion. If improvement is only transient a second infusion of gum may be administered and is frequently followed by steady improvement. If even after this the patient does not improve, blood transfusion should be given a trial.

The third group of "uncompensated" cases are those in which the pulse cannot be felt and the blood pressure has fallen below 60 mm. mercury. The heart rate is found on auscultation to be 120 to 180. Some cases have a heart rate below 100 but this is almost invariably a terminal phenomenon. The blood volume is below 65 per cent of the normal. The patient is in an extremely serious condition. He is restless, very thirsty and vomits immediately on being given fluid. The extremities are very cold to touch. The pulse cannot be felt. In this third group infusion of gum solution and even transfusion of whole blood are as a rule without avail.

The gum acacia solution used by Keith consists of 6 per cent solution. It may be advisable to try the concentration used experimentally by White and Erlanger consisting of 18 per cent glucose and 25 per cent gum acacia.

The experiments of Aub and his co-workers suggest that the vicious circle of anorexia should be combated with plenty of fresh air or the administration of oxygen.

During the war when cases of shock requiring treatment were very numerous, the availability of gum acacia solution rendered its use highly desirable. But in peace time cases of shock are usually single and usually there is ample opportunity for obtaining sufficient whole blood for the transfusion. There have been many cases in which gum acacia even when used as outlined by Bayliss has been without avail. This of course is also true of blood transfusion, but apparently less frequently. It appears that trans-

fusion is the method of choice whenever practicable. Whole blood may be used or if transfusion is repeated frequently, blood plasma without the corpuscles may be used.

There is considerable difference of opinion as to whether direct transfusion or indirect, with the use of sodium citrate, is to be preferred. A great disadvantage of the latter method is that in a very high proportion of cases so treated a reaction occurs in the form of chill and frequently fever. In some cases the added burden of this reaction might be sufficient to determine a fatal issue. With the most painstaking administration one is never entirely sure whether or not citrated homologous blood is going to produce a reaction. Direct transfusion, when correctly performed is free from this disadvantage.

Stimulation of the heart with digitalis preparations or the vasomotor system with strychnine appears not to be indicated by the evidence.

Ether anesthetization certainly predisposes to shock. When there is danger of shock gas oxygen or local anesthesia should be used if possible, or a minimum of ether should be administered. The abundant administration of fluid by mouth in pre-operative cases is strongly indicated by the experimental evidence. It may be that at some future time some substance will be discovered which will neutralize the poisonous action of the hypothetical histamine-like body.

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—W. T. V.

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## ORIGINAL ARTICLES

### CHAULMOOGRA OIL IN THE TREATMENT OF TUBERCULOSIS\*

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THE use of chaulmoogra oil in the treatment of leprosy and various entaneous diseases has been recorded since 1596 A.D. The oil is obtained from the seeds of *Taraktogenos Kurzii*, King Family, Flacourtiaceae, a tree native to the forests of Burma and eastern India. The tree produces a large fruit with numerous seeds imbedded in the pulp and it is from these seeds that the oil is expressed. The term "*Chaulmoogra odorata*" was applied to the tree by Roxburgh in 1814. Siam produced a tree closely related to the gynocardia, the seeds of which were used by the ancient Chinese for medicinal purposes.

The crude oil, expressed cold from the seeds, is a solid, possessing light brown color, pungent odor, highly disagreeable and nauseating taste. The early method of administration was by external application in conjunction with other oils. This was superseded by an oral administration. The nauseating and irritating properties have been an obstacle to its use in modern medicine. The problem presented was that of retaining the active principle of the oil and at the same time eliminating the undesirable effects. Little progress was made until 1890, when hope of a possible cure for leprosy aroused by the heroic efforts of the Belgian priest, Father Damien, stimulated further investigation with chaulmoogra oil.

The oral administration of the drug was unsatisfactory because of the unavoidable gastric disturbance which accompanied it. Investigators accordingly resorted to subcutaneous and intramuscular injection. Encouraging results were obtained by mixing the oil with camphor and resorcin which reduced its irritating properties and rendered it more fluid. In Dyer's opinion the drug was most efficacious when combined with camphor and resorcin.

Hollman and Dean recently isolated the fatty acids of chaulmoogra oil

\*From the Research Laboratory, Parke, Davis & Co., Detroit, Mich.

separating them into groups by fractional crystallization. They converted the individual groups or fractions into each respective ethyl ester in which form they used the drug in treating leprosy. Marked improvement was reported in cases treated over a period of four months, with these fractions.

Sir Leonard Rogers was the first to employ the soluble sodium and potassium salts of chaulmoogra oil. His investigations conducted in 1915-16-17 using the salts orally and intravenously gave exceedingly promising results, a cure being effected in some cases and distinct improvement in all patients treated.

Walker and Sweeney reported their findings in an investigation in March, 1920. They determined that the action of chaulmoogra oil is bactericidal rather than physiologic, the active principle is the fatty acids; the action is specific for acid-fast bacteria and is a function of the carbon ring structure of the molecule of chaulmoogric acids found only in chaulmoogra oil and oils of trees closely related to the *gynocardia odorata*. They suggested that it may be of benefit in the treatment of tuberculosis and are conducting investigations to determine this point.

Numerous investigators have apparently come to the same conclusion with reference to the treatment of leprosy with chaulmoogra oil or its active principles, i. e., a high percentage of patients are improved and a few cures are recorded. The similarity in morphology and staining characteristics of leprosy bacilli and tubercle bacilli and the specific action of chaulmoogra and its related oils on the acid-fast group of bacteria, furnish a scientific basis for using them in the treatment of tuberculosis. With the following objects in view our work was started early in January, 1920. The acid fractions of chaulmoogra oil obtained by the method of Hollman and Dean and modified by T. B. Aldrich were used in the first attempt. T. B. Aldrich of this institution prepared all the fractions used throughout the investigation.

The scope of our efforts is as follows:

1. To determine the most active, soluble and least irritating fraction obtained from the oil.
2. To determine the most effective method of administration.
3. To determine what pathological effect, if any, is induced by large doses of the fractions of the oil.
4. To determine the bactericidal properties for tubercle bacilli *in vitro*.
5. To determine whether its use will inhibit or arrest the development of artificially induced tuberculosis in guinea pigs.

Fifty healthy guinea pigs, male and female, averaging about 500 grams were selected. In about two weeks the experiment was terminated when an epidemic of bronchisepticus and pneumonia appeared in our animal house. So many pigs were lost from these two diseases that it was necessary to select another group of fifty and repeat the experiment. The only definite and positive information gathered from our first group of fifty pigs, was, that the ethyl esters were exceedingly irritating when administered intraperitoneally and slightly toxic in l. c. c. doses; that the peritoneum showed chronic inflammation, particularly at the point of injection. The spleen and liver were hypertrophied and showed beginning fatty degeneration; that the fatty acids were

very insoluble; that a virulent suspension of the tubercle bacilli incubated 48 hours at 37° C. with each of the acid fractions in a dilution of 1:10,000 failed to kill any of the twelve pigs infected or to grow when transplanted.

The second group of fifty pigs were selected February, 1920, and the work started as before. We used the acid sodium salts of chaulmoogra oil to replace the ethyl esters. Nine days from the date the investigation was started a second epidemic was encountered and so many pigs were lost that it was necessary to discard the remaining and select another group of fifty. The information gathered from this attempt, was that the acid sodium salts of the four fractions, A, B, C, and D, of chaulmoogra acid are fairly soluble and far less irritating when administered intraperitoneally. They were not toxic in 1 c.c. doses.

A third group of forty-eight pigs was begun on July 27 under most favorable conditions. We used a large concrete building 20x40x16 ft., with 14 ordinary sized windows and one door. This room was equipped with one dozen 4x2 pens which were well ventilated. This place was made to assume, as near as possible, the proportions, conditions, and surroundings of the loft where the pigs were raised. They were given an abundance of the same foods that they had always received. The pens were kept clean and the water receptacle full of fresh water at all times. The pigs were kept free from all body vermin. The soluble acid sodium salts of the four acid fractions respectively were prepared. We used a 1 per cent solution of each of the salts of the fractions prepared according to the methods of Hollman and Dean, Walker and Sweeney and modified by Aldrich.

On July 27 the twenty-four pigs were inoculated with 0.5 c.c. of a heavy suspension of virulent tubercle bacilli isolated by the writer from a patient suffering with pulmonary tuberculosis. Twelve of these pigs were selected and designated as Group I or known as our tuberculous controls. These pigs were placed in two pens, six in each and not molested further. The remaining twelve of the twenty-four inoculated with tubercle bacilli were designated as Group II. This group was subdivided into four groups of three pigs each and the first subdivision containing three pigs was designated as fraction "A" group. Each of these animals received six 0.2 c.c. doses of the "A" fraction of the acid sodium salt of chaulmoogra oil intraperitoneally, at three day intervals, and nine 0.3 c.c. doses, administered the same way at the same intervals. The second subdivision, containing three pigs was designated as fraction "B" group. Each of these animals received six 0.2 c.c. doses of the "B" at three day intervals, and nine 0.3 c.c. doses administered the same way at the same intervals. The third subdivision containing three pigs was designated as fraction "C" group. Each of these animals received six 0.2 c.c. doses of the "C" at three day intervals, and nine 0.3 c.c. doses administered the same way at the same intervals. The fourth subdivision containing three pigs was designated as fraction "D" group. Each of these animals received six 0.2 c.c. doses of the "D" fraction of the acid sodium salt of chaulmoogra oil, intraperitoneally, at three day intervals, and nine 0.3 c.c. doses administered the same way at the same intervals.

A third group of eight pigs was selected and designated as Group III or our normal controls. They were placed in two pens, four in each and not

molested further except they were weighed every third day. The object in retaining this group of animals was to have a control for the living conditions.

A fourth group of twelve pigs was selected and designated Group IV, being placed in four pens, three in each pen. Each of the three pigs in pen (A) received six 0.2 c.c. doses of the "A" fraction of acid sodium salts of chaulmoogra oil intraperitoneally at three day intervals and nine 0.3 c.c. doses administered the same way at the same intervals. This group of pigs was designated fraction "A" group not infected with tubercle bacilli. These animals were used as a check against our subdivision of Group II, fraction "A" group infected with tubercle bacilli. Each of the three pigs in pen "B" received six 0.2 c.c. doses of "B" fraction of the acid sodium salts of chaulmoogra oil intraperitoneally at three day intervals and nine 0.3 c.c. doses administered the same way at the same intervals. This group of pigs was designated Fraction "B" group not infected with tubercle bacilli. These animals were used as a check against our subdivision of Group II, fraction "B" group infected with tubercle bacilli. Each of the three pigs in pen "C" received six doses 0.2 c.c. of "C" fraction of the acid sodium salt of chaulmoogra oil intraperitoneally at three day intervals. This group of pigs was designated fraction "C" group not infected with tubercle bacilli. These animals were used as a check against our subdivision of Group II fraction "C" group infected with tubercle bacilli. Each of the three pigs in pen "D" received six 0.2 c.c. doses of fraction "D" of the acid sodium salt of chaulmoogra oil intraperitoneally at three day intervals and nine 0.3 c.c. doses administered the same way at the same intervals. This group of pigs was designated fraction "D" group not infected with tubercle bacilli. These animals were used as a check against our subdivision of Group II, fraction "D" group infected with tubercle bacilli.

A fifth group of four pigs was selected and placed in a pen. These pigs were the toxicity and quantity test animals. One pig was selected, tagged and marked "A," another was tagged and marked "B," another "C" and the last "D." Pig "A" received 0.5 c.c. of fraction "A" of the acid sodium salt of chaulmoogra oil intraperitoneally at three day intervals until a total of 4 c.c. was administered. Pigs "B," "C" and "D" were treated the same way, using fraction "B," "C" and "D" of the acid sodium salt of chaulmoogra oil respectively for pigs "B," "C" and "D." These animals were autopsied 24 hours after they had received the injection.

This accounts for the disposition of the original 48 pigs which were used in the investigation.

To make it possible to draw a comparison, from a pathologic standpoint, between our Group I (tuberculous controls) and our Group II (treated pigs infected with tuberculosis) it was necessary to have a constant factor. We chose time as this factor. It was, therefore, necessary for us to kill a pig from Group II (treated pigs infected with tuberculosis) when a pig from Group I (tuberculous controls) died. This system kept the number of days lived by each group uniform and allowed us to study the gross pathology at autopsy, if any, and supplied us with material for microscopic study. Our next step was to devise a scaling system which would also be constant

for both Groups I and II. The following was used for making counts. Before giving the scaling outline the writer wishes to state that he autopsied and scaled the animals first, keeping this as a permanent record, then he removed all marks of identification from the animal and his assistant was allowed to do the scaling. All throughout the investigation there was never more than a difference of one between our counts and often times our totals were the same. We may, therefore, assume that this factor was constant enough to give us grounds for basing an opinion on one phase of the investigation.

#### PATHOLOGIC SYSTEM OF SCALING

Glandular Involvement	<ol style="list-style-type: none"> <li>1. Glands enlarged but no nodules visible.</li> <li>2. Glands enlarged and few nodules present.</li> <li>3. Glands enlarged and all axillary glands showing nodules or tuberculous growth.</li> </ol>
Peritoneal wall	<ol style="list-style-type: none"> <li>1. Granular appearance with a few scattered tubercles.</li> <li>2. Tubercles more thickly distributed, but still discrete.</li> <li>3. Tubercles coalescing, forming solid greyish furry coat on peritoneum, or heavy cheesy crust.</li> </ol>
Diaphragm	
Mesentery	
Spleen	<ol style="list-style-type: none"> <li>1. Granular or slightly enlarged, no tubercles visible.</li> <li>2. Enlarged, congested, small tubercles scattered throughout tissue.</li> <li>3. Enormously enlarged, large coalescing areas, caseous or softening with presence of pus, or cheesy areas.</li> </ol>
Pancreas	<ol style="list-style-type: none"> <li>1. Somewhat enlarged.</li> <li>2. Enlarged, showing tuberculous nodules and beginning to harden.</li> <li>3. Very large and hard, nodules very prominent, adhesions to adjacent tissues.</li> </ol>
Liver	<ol style="list-style-type: none"> <li>1. Not enlarged but appearing either slightly granular in consistency or mottled in color.</li> <li>2. Slightly enlarged with minute discrete tubercles apparent.</li> <li>3. Enlarged, tubercles coalescing, or caseous areas or soft granular areas or both.</li> </ol>
Lungs	<ol style="list-style-type: none"> <li>1. Slightly granular or faint pearly areas. Appearance decidedly not normal.</li> <li>2. Tubercles definitely present but small and widely scattered or confined to one lobe or portion of a lobe.</li> <li>3. All tissue heavily studded with coalescing nodules, congested areas and pus present.</li> </ol>
Fluids	<ol style="list-style-type: none"> <li>1. Slight excess in either or both body cavities.</li> <li>2. Large excess in either cavity.</li> <li>3. Excessive fluids or pus in both cavities.</li> </ol>
Kidney	<ol style="list-style-type: none"> <li>1. Capsule or outer membrane of one or both kidneys showing tubercles.</li> <li>2. Capsule showing many tubercles, surface beneath mottled or corrugated.</li> <li>3. Capsule showing many tubercles, also upon sectioning tubercles exhibited.</li> </ol>

#### SUMMARY

One per cent solutions of the soluble acid sodium salt of the four acid fractions of chaulmoogra oil were apparently the most active, soluble and least irritating when administered hypodermatically. There was a conspicuous absence of the drug in the peritoneal cavity of an animal when it came to autopsy although less than 24 hours had elapsed after an heroic dose.

Charts "D" and "E" show a marked gain in weight for the animals which received the chaulmoogra salts, probably indicating their complete

## GROUP I PIGS (TUBERCULOUS CONTROLS)

PIG IDENTIFICATION	DATE OF INOCULATION	AMOUNT OF VIRULENT HUMAN TUBERCLE USED	WEIGHT OF PIG AT INOCULATION	WEIGHT AT AUTOPSY	DAYS LIVED	PATHOLOGY COUNT SCALED	L = LIVING D = DIED K = KILLED
7—A	July 27-20	0.5 c.c.	440	360	48	21	D
7—B	" " "	"	350	320	28	22	D
7—C	" " "	"	410	295	74	20	D
7—D	" " "	"	510	430	40	16	D
7—E	" " "	"	475	385	40	21	D
7—F	" " "	"	500	395	42	26	D
8—A	" " "	"	375	340	78	20	D
8—B	" " "	"	520	585*	85*		L
8—C	" " "	"	380	220	29	19	D
8—D	" " "	"	395	325	41	21	D
8—E	" " "	"	490	445	48	19	D
8—F	" " "	"	420	495	37	21	D
Average		0.5 c.c.	438.75	382	49	20	

Average loss in weight during the 49 days lived is 56 grams

\*Pig is living on this date.

## GROUP II PIGS (INFECTED WITH HUMAN TUBERCULOSIS AND TREATED)

PIG IDENTIFICATION	DATE OF INOCULATION	AMOUNT OF VIRULENT HUMAN TUBERCLE USED	WEIGHT OF PIG AT INOCULATION	WEIGHT AT AUTOPSY	DAYS LIVED	PATHOLOGY COUNT SCALED	TOTAL AMOUNT OF 1% ACID SODIUM SALT OF CHAULMOOGRA ADMINISTERED	TOTAL NUMBER OF TREATMENTS	L = LIVING D = DIED K = KILLED
1—A	July 27-20	0.5 c.c.	525	625	85*		3.9 c.c.	15	L
1—B	" " "	"	455	420	40	15	3 "	12	K
1—C	" " "	"	375	350	42	15	3.9 "	15	K
2—A	" " "	"	325	275	40	11	2.4 "	10	K
2—B	" " "	"	315	270	48	16	3.3 "	13	K
2—C	" " "	"	560	470	74	13	3.9 "	15	K
3—A	" " "	"	320	410	78	13	3.9 "	15	K
3—B	" " "	"	405	355	28	9	1.8 "	8	K
3—C	" " "	"	415	440	48	15	3.9 "	15	K
4—A	" " "	"	420	370	29	10	2.1 "	9	K
4—B	" " "	"	370	285	37	12	2.4 "	10	D
4—C	" " "	"	480	410	41	17	3.9 "	15	K
Average			413.75	390	49	13.33	3.2	12.6	

Average Loss in weight for 49 days Lived is 23 grams

\*Pig is living on this date.

assimilation. Basing our opinion on the findings in Group II we believe fraction "A" and "B" most potent.

No pathologic findings which could be attributed to the drug were found at necropsy of pigs receiving the chaulmoogra salts, as exhibited by Group V pigs. These pigs gained weight during the administration of the drug. A 1 per cent solution of the acid sodium salts of all the acid fractions of chaulmoogra oil we used were found to be nontoxic as shown by the fact



CHART FOR COMPARING GROUP I WITH GROUP II

PIG IDENTIFICATION	GROUP NO.	DAYS LIVED	LOSS OR GAIN IN WEIGHT	PATHOLOGY COUNTS SCALED	NET POINTS CREDITED GR. II IN WEIGHT	NET POINTS CREDITED GR. I IN COUNTS	NET POINTS CREDITED GR. I IN WEIGHT	NET POINTS CREDITED GR. I IN COUNTS	NET POINTS DEBITED GR. II IN WEIGHT	NET POINTS DEBITED GR. II IN COUNTS	NET POINTS DEBITED GR. I IN WEIGHT	NET POINTS DEBITED GR. I IN COUNTS
1-A	II	85*	+100 <sup>7</sup>		35							
2-B	I	85*	+ 65*							35		
1-B	II	40	- 35	15	10	6						
7-E	I	40	- 45	21						10	6	
1-C	II	42	- 25	15	80	5						
7-F	I	42	-105	20						80	5	
2-A	II	40	- 50	11	30	5						
7-D	I	40	- 80	16						30	5	
2-B	II	48	- 45	16		5			10			
7-A	I	48	- 35	21			10				5	
2-C	II	74	- 90	13	25	7						
7-C	I	74	-115	20						25	7	
3-A	II	78	+ 90	13	125	7						
8-A	I	78	- 35	20						125	7	
3-B	II	28	- 50	9		13			20			
7-B	I	28	- 30	22			20					13
3-C	II	48	+ 25	15	70	4						
8-E	I	48	- 45	19						70	4	
4-A	II	29	- 50	10	110	9						
8-C	I	29	-160	19						110	9	
4-B	II	37	- 85	12		9			160			
8-F	I	37	+ 75	21			160					9
4-C	II	41	- 70	17		4						
8-D	I	41	- 70	21								4
Average		49.1			40	6						

\*Pigs living on this date.

that no pigs were lost from groups IV or V; on the other hand all showed a marked increase in weight after the administration of heavy doses. Group IV curve suggests the presence of a vitamine or some element which has a marked influence on the weight of the pigs.

The peritoneal administration in the case of guinea pigs was found to produce no undesirable effects. In this case peritoneal administration was found to be a method by which the salts may be rapidly absorbed by the body.

We found the acid sodium salt of Chaulmoogra oil has a specific bactericidal action on tubercle bacilli. Sweeney and Walker showed it was specific for the acid-fast group in a 1:100,000 dilution. We were only able to prove its bactericidal properties for tubercle bacilli in a dilution of 1:10,000.

Of the 12 pigs inoculated with tuberculosis and not treated, all died except one, of the 12 pigs treated only one died.

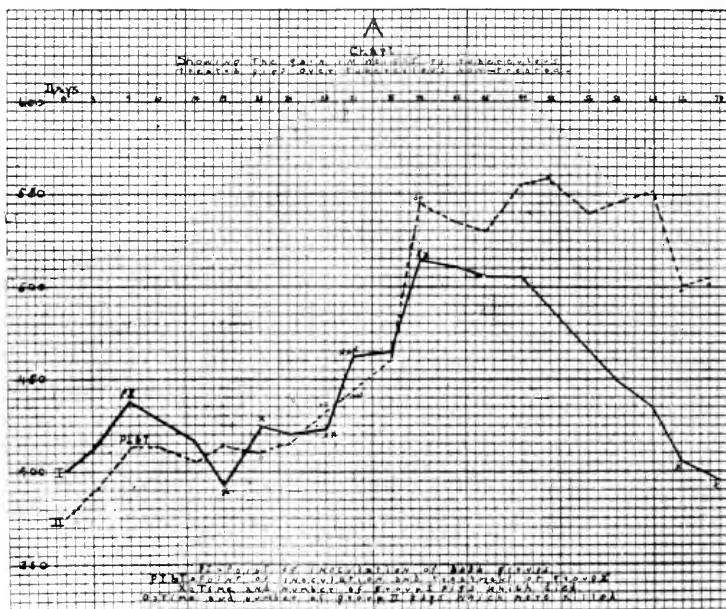
A marked difference in the pathologic findings between pigs which were and were not treated was observed, the advantage being in favor of the treated pigs.

Treated pigs showed an average gain of 49 grams over the ones not treated; the time factor being kept constant.

One pig from Group II which was treated with fraction "A" gave birth to two young ones after the 18th treatment.

More evidence in favor of Group II as compared to I will be found upon close observation and comparison of Charts A, B, and C. It will be noted in Charts A, B and C that at the close of the investigation there is a distinct advantage Group II shows over Group I.

We have more work under way to verify these findings inasmuch as this is a preliminary report.



CONSIDERATION OF CHART A

Chart A is based on the fact that when a pig in Group I died an animal from Group II was killed and the average weight of the remaining animals in each group recorded by the respective curves.

We find the following factors most prominent when Group I pigs (tuberculous controls) are considered.

1. That the average group gain for the first seven days was normal when compared with our normal controls.
2. From the seventh (the point of inoculation) to the seventeenth day the average loss in weight was 43 grams per pig.
3. From the seventeenth to the thirty eighth day the average gain per pig in the group was 115 grams. This is explained by the fact that the light weight pigs were first to die.
4. From the thirty-eighth to the seventieth day the average loss per pig in the group was 115 grams.

5. From point of inoculation to end of investigation a loss of 43 grams per pig was sustained.

We find the following factors most prominent when Group II pigs (infected with tuberculosis and treated) are considered.

1. The average gain for this group for the first seven days was normal when compared with our normal controls.

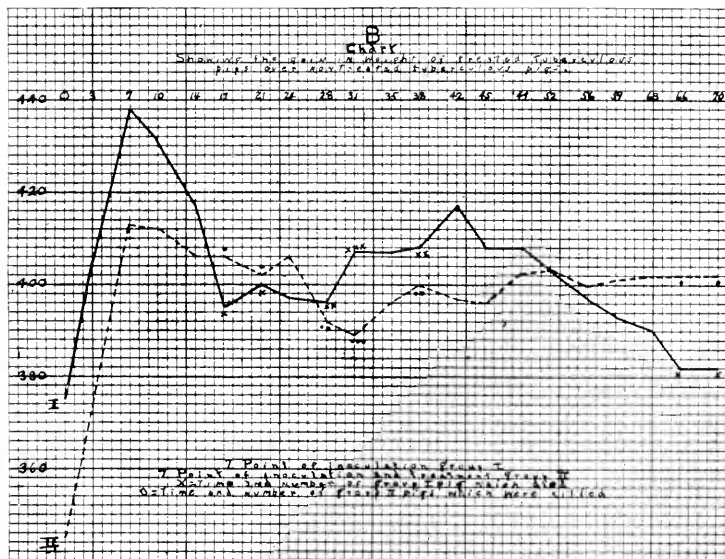
2. From the seventh (point of inoculation and treatment) to the seventeenth day the average loss in weight was two grams, which is not abnormal.

3. From the seventeenth to the thirty-eighth day there was an average gain of 134 grams per pig. This is partially explained by the fact that we tried to select pigs to kill whose weights approximated those of the dead pigs of Group I.

4. From the thirty-eighth to fifty-second day there was an average group gain of 11 grams.

5. From the fifty-second to the seventieth day there was an average loss of 51 grams per pig.

6. From the seventh to seventieth day an average gain of 92 grams per pig.



CONSIDERATION OF CHART B

In drawing Chart B the weight of each pig at necropsy which died or was killed was retained as a constant factor throughout the remainder of the investigation. The points on the chart were the average weights for the entire twelve pigs of each respective group.

The following points on this chart appear most interesting when Group I pigs (tuberculous controls) are considered.

1. The entire group individually and as a unit lost weight uniformly and gradually.

2. The gain in group weight during the first week coincided with the gain in weight of the other normal groups.

3. This group started with each pig averaging 25 grams more than Group II, at point of inoculation.

4. Each animal in the group lost an average of 43 grams the first ten days after inoculation. This is probably due to the virulence of the organism used.

5. All animals in the group died except one.

6. A total loss of an average of 56 grams per pig was sustained from the date of inoculation to the end of the investigation.

7. The pathology count sealed for this group averages 20 per pig.

*Consideration of Group II pigs infected with tuberculosis and treated.*

1. The entire group individually and as a unit lost weight uniformly and gradually but the amount lost could not be considered abnormal.



2. The gain in group weight during the first week coincides with the gain in the weight of the normal group.

3. This group started with each pig averaging 25 grams less than Group I. This group overcomes this handicap throughout the investigation.

4. Each animal in this group lost an average of 7 grams (which is not considered an abnormal fluctuation) the first ten days after inoculation.

5. All animals in the group were killed except one.

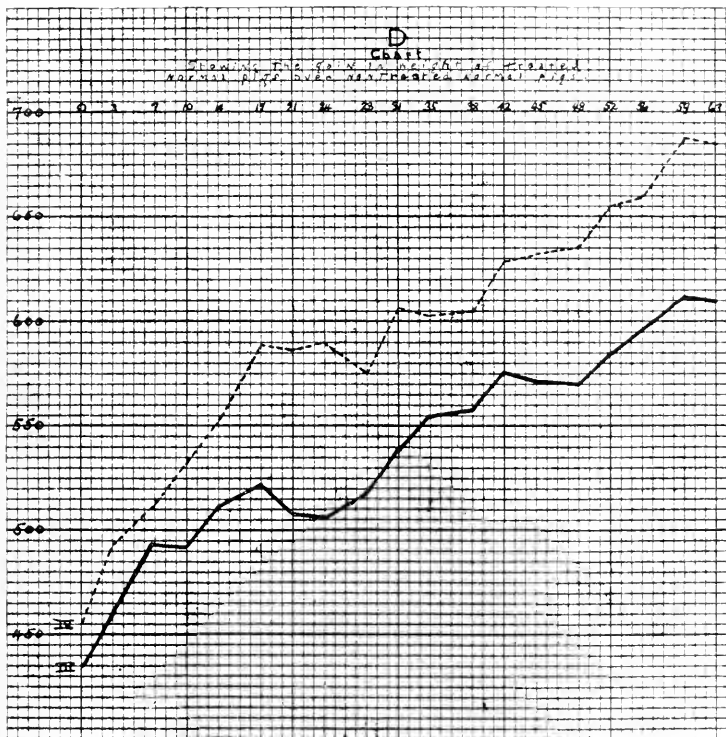
6. The highest point reached by this group was an average of 406 grams.

7. A total loss of an average of 11 grams per pig was noted from the point of inoculation to the end of the investigation.

8. The pathology count sealed for this group averages 13.33 per pig.

## CONSIDERATION OF CHART C

In drawing Chart C the weight at necropsy of the pigs which died or were killed was retained as a constant factor throughout the remainder of the investigation. The points on the chart represent the average loss or gain for the entire twelve pigs based on the original weight of each respective group. Example, if Group 1's original weight is 413 grams per pig and at the period of the third weighing is 406 grams this group sustains an average loss of 7 grams per pig over a period of ten days.

*Consideration of Group I pigs (tuberculous controls)*

1. From point of inoculation, which is charted zero, to the tenth day there was a loss of 43 grams per pig.
2. From the tenth to the thirty-fifth day there was a gain of twenty-two grams per pig.
3. From the thirty-fifth to the sixty-third day there was a loss of thirty-seven grams per pig.
4. From zero to the sixty-third day there was a loss of an average of 58 grams per pig for the group.
5. The entire group lost weight sporadically.

*Consideration of Group II Pigs (infected with tuberculosis and treated)*

1. From point of inoculation, which is charted zero, to the tenth day, there was a loss of eight grams.
2. From the tenth to the thirty-fifth day there was a loss of eight grams.
3. From the thirty-fifth to the sixty-third day there was a loss of seven grams.
4. From zero to the sixty-third day there was a loss of nine grams.
5. The entire group lost weight uniformly and gradually.

## CONSIDERATION OF CHART D

In drawing Chart D the average weights at successive weighing periods of the entire number of pigs of each respective group were recorded as points on the chart, eight pigs being used for Group III and twelve for Group IV.

*Consideration of Group III Pigs (Normal controls)*

1. None of these pigs died during the course of the investigation.
2. Individually and as a group the pigs showed a steady and gradual increase in weight from the date of inoculation of Groups I and II to the end of the investigation.
3. The entire average gain in weight for the group is 176 grams.
4. This group weighed at start twenty-one grams less than Group IV, and at the end of the investigation 75 grams less than Group IV.
5. The average gain for a three day period was 9.7 grams.

*Consideration of Group IV Pigs (Injected with chaulmoogra oil at same periods and in same amounts as pigs of Group II)*

1. None of these pigs died during the course of the investigation.
2. All showed a steady and marked increase in weight from the date of first treatment to the end of the investigation.
3. The entire average gain in weight for the group is 230 grams.
4. The group weighed at start 21 grams more than Group III and at the end of the investigation 75 grams more, a gain per pig of 54 grams over the normal pigs.
5. The average gain for a three-day period was 12.7 grams.

The authors wish to thank Dr. E. M. Houghton, at whose suggestion this work on chaulmoogra oil was undertaken and for his kind criticisms and suggestion in carrying it out. Also, we wish to acknowledge the assistance given by Mr. Clark.

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# THE PHARMACOLOGIC ACTION OF LEAD IN ORGANIC COMBINATION<sup>\*</sup>

BY E. C. MASON, M.D., CINCINNATI, OHIO

## INTRODUCTION

THE frequent occurrence of lead poisoning, accompanying the extensive use of lead compounds in the various arts and trades, has made the subject of "Lead Poisoning" one of wide-spread interest. This is evidenced by the fact that one of the oldest works on Trade Hygiene (by Stockhansen) is entitled, "*De lithargyrii fumo noxio, morbifico ejusque metallico frequentiori morbo vulgo dicto hüttenkatze*," Gaslar, 1556.

The deaths occurring from lead poisoning in England and Wales during the ten years 1883 to 1892 were no less than 1043.<sup>1</sup> Of this number three were suicidal; the remaining 1040 were mainly from the manufacture of white lead and from the use of lead in the arts, or from accidental contamination of foods. For the ten years<sup>2</sup> ending 1909, 8,973 cases of lead poisoning with 667 deaths were reported to the Home Office (England) as occurring in 18 industries, but Legge points out that there has been in this period a reduction of more than 50 per cent of cases. It is difficult to obtain similar data in the United States, but Alice Hamilton<sup>3</sup> reports 358 cases with 16 deaths in 23 white lead factories during the 16 months prior to May 1, 1911. In New York State in 1909 and 1910,<sup>4</sup> 60 deaths were certified from lead poisoning. These figures represent but a small percentage of the individuals who actually suffered from lead poisoning, as the percentage of deaths is relatively low.

In spite of the enormous number of observations made and recorded by various investigators, the mode of action of lead on the body has not been definitely determined. The reason for this is quite evident; being due to the fact that the compounds of lead usually available for experimental purposes, have been, for the most part, such as could not be administered intravenously. Therefore, our knowledge of this subject has been limited mainly to clinical observations.

## CLINICAL SYMPTOMS OF LEAD POISONING

Clinically lead poisoning is recognized as being of two types; viz., acute and chronic. The characteristic symptoms of *acute poisoning* develop after the ingestion of a considerable dose of some such salt as "sugar of lead," and usually appear within a few minutes; there is noted immediately a sweet,<sup>5</sup> then a disagreeable metallic and astringent taste, with burning, and a sensation of great dryness in the mouth and throat. Vomiting usually occurs within fifteen minutes, but in rare cases it may be delayed from one to two

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hours. The retching and vomiting are very obstinate, and continue for a long time; the vomitus is often milky with lead chloride and sometimes streaked with blood; there is pain in the abdomen of a colicky character, also thirst. The bowels are as a rule constipated, but occasionally there is diarrhea with black stools (lead sulphide). The urine is generally diminished. The breath has a foul odor, and the tongue is coated; the skin is dry, and the pulse small and frequent. In addition there may be present various nervous manifestations; such as, headache,<sup>8</sup> shooting pains in the limbs, cramping in the legs and local numbness. Fatal cases pass into coma and die with or without convulsions.

The characteristic symptoms of *chronic lead poisoning* are those of general ill health. The symptoms may at first be indefinite, coming on irregularly and often months after the individual has discontinued working with lead. There are general digestive disturbances, loss of appetite, nausea, vomiting, diarrhea or constipation. There are generally present certain blood changes, the number of red cells and the hemoglobin index being low. The red cells, when stained with methylene blue show basophilic granules. This "stippling" is one of the important and almost constant diagnostic signs. Schnitzer<sup>8</sup> attributes these blood changes to injury of the bone-marrow.<sup>9</sup> The skin assumes a peculiar yellowish hue, and in some cases the patient is distinctly jaundiced. This is due to the wide-spread destruction of the red corpuscles. The gums show a black line which has been demonstrated to be lead sulphide. The symptoms which often develop later are highly characteristic and assume various forms; in the order of their frequency there may appear colic, arthralgia, paralysis, nephritis, cerebral symptoms and anesthetics. Of Tanqueril's cases<sup>10</sup> there were 1217 of colic, 101 of paralysis and 72 of encephalopathy.

One of the best pieces of work ever done on experimental lead poisoning was that of Harnack<sup>11</sup> published in 1878. Harnack used lead in organic combination in the form of the triethyl lead acetate, and his observations were made on frogs, rabbits, cats and dogs. The work consisted mainly in administering the lead compound to these animals, either subcutaneously or intravenously, and observing the symptoms which developed. These observations represent a very valuable contribution to our knowledge of lead poisoning, and, considering the date at which the experiments were performed, it is indeed a splendid piece of work. There appear to be, however, many parts of Harnack's work of which further study would be exceedingly desirable. Therefore, I have continued the investigation of this subject, using the following preparations: *Triethyl lead, triethyl lead chloride, triethyl lead acetate, trimethyl lead, trimethyl lead chloride and triethyl antimony nitrate.*

#### PREPARATION OF COMPOUNDS

These preparations were first made by Löwig<sup>12</sup> (1853) who prepared the triethyl lead, and in addition the oxide, carbonate, sulphate, nitrate, chloride and bromide of the compound. The work of Löwig was repeated by Klippel<sup>13</sup> (1860) who, in addition, made the following compounds: the phosphate, formate, acetate, butyrate, benzoate, tartrate, oxalate, sulphide, cyanide, sulphocyanate and also double salts with mercury chloride and with platinum chloride.

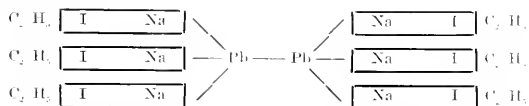


He also repeated some of the work, using amyl iodide instead of ethyl iodide. As previously stated, Harnaek (1878) made the acetate and studied its action on frogs, rabbits, cats and dogs.

I have prepared these triethyl lead compounds according to the method used by Löwig and Klippel with various modifications.

As a starting point for the preparations, it is necessary to obtain a sodium-lead alloy. This alloy is made by melting 50 grams of lead, and introducing into the molten mass 16 grams of sodium, which has been previously cut into rather small pieces, about 1 cubic centimeter in size. The reason for selecting 50 grams of lead is that it gives a yield of a desirable amount, while the reason for combining lead and sodium in a ratio of 50 to 16 is based on their atomic weights (lead 206.9, sodium 23.05), the product desired being an atomic relation of  $\text{Pb}(\text{Na})_3$ .

After the sodium and molten lead are united, a short time should be allowed to elapse until the mass starts to form a crust over the surface. At this point it should be covered with hot, dry sand and allowed to cool. When cool the mass is best ground in an iron mortar, and when reasonably well powdered, it is put in an Erlenmeyer flask of about 200 c.c. capacity. The flask is connected with an upright, water-cooled condenser. Through the upper end of the condenser is introduced 50 c.c. of ethyl iodide or ethyl bromide. A reaction should take place, lasting for two or three hours. If reaction fails to take place, 1 or 2 c.c. of ethyl alcohol may be added. The reaction is as follows:

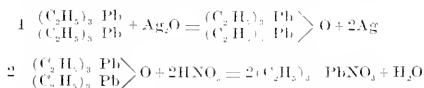


When the reaction is complete, the excess ethyl iodide or ethyl bromide is distilled off, and the contents of the flask is extracted with ether. This is best done by nearly filling the flask with ether and stirring the contents with a stirring rod. The mixture is then allowed to settle and the ether extract is poured into a graduated cylinder, which should be large enough to contain three such extractions. This extraction process is repeated twice. The extract, after the suspended matter has settled, should be clear and have a greenish-yellow color. It is then poured from the graduate into a distilling flask of one liter capacity, and about 200 c.c. of water is added to the contents. The ether is then distilled off on a water bath, and the compound, being insoluble in water, collects as an oily mass beneath the water in the flask.

This oil has a specific gravity of 1.471 at  $10^\circ \text{C}$ ., and corresponds to the formula of  $(\text{C}_2\text{H}_5)_3\text{Pb-Pb}(\text{C}_2\text{H}_5)_3$ . The compound is but slightly soluble in water, not very soluble in alcohol, but quite soluble in ether.

The salts of the compound may be made by one of two methods; first, by preparing the hydroxide and neutralizing with the acid, or, second, the method introduced by Klippel, which consists in making the desired salt from the carbonate. The first method is the one I have used. The oil is treated in a beaker (with enough alcohol to cover it well) with an alcohol solution of

silver oxide made acid by nitric acid. (The concentrated nitric acid should be diluted with one-half its volume of water, and this nitric acid solution is added until only a small amount of silver oxide is present as such in the alcohol solution.) This alcoholic solution of silver oxide and nitric acid is added to the alcoholic solution of the oil, slowly and with constant stirring until metallic silver no longer precipitates out. The alcoholic solution is then filtered and contains the nitrate of the compound in solution.

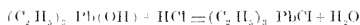


The solution is then neutralized with an alcoholic solution of sodium hydroxide. A precipitate often occurs at this point, which is probably a basic compound of the lead or silver and should be removed by filtration. The hydroxide of the compound remains in the alcoholic solution.



To obtain the hydroxide in a more purified form, one may treat this alcoholic solution with ether and much water. The water and alcohol are completely miscible which forces the compound into the ether layer. The ether layer is separated from the water by means of a separatory funnel, and the ether layer, after distillation, yields a thick mass, which, on standing, becomes somewhat crystalline.

If the alcoholic solution of the hydroxide is treated with dilute hydrochloric acid, the chloride of the compound will, in the course of time, crystallize in long, beautiful crystals, having a silky luster.



In the course of the analysis of these compounds, they were heated, as is the usual custom, to 105° C. to drive off the moisture. However, it was found impossible to obtain a constant weight, and for that reason it was supposed that decomposition was taking place. Therefore, instead of heating samples to 105° C., the temperature was maintained at 95° C. The following analysis will show the result of such heating:

Acetate No. III, dried over sulphuric acid for one month, was heated to 95 degrees.

.2476 g. lost .0473 g. or 19.10 per cent.

.2135 g. lost .0624 g. or 29.22 per cent. The heating was discontinued at this point and the samples analyzed.

*Analysis of the Dried Acetate.*

.2003 g. gave .1708 g. Lead Sulphate or 58.21 per cent.

.1511 g. gave .1283 g. Lead Sulphate or 58.00 per cent. Theoretical 58.62 per cent.

It is evident from the above figures that these compounds undergo sublimation when heated to such temperatures as those indicated. This analysis was made on a preparation which had been prepared in the early part of the

work, and which was not subjected to purification by recrystallization from alcohol. However, the analysis shows a fair degree of purity.

The lead in these compounds is in the masked or protected form, which prevents the lead from reacting with the usual precipitating reagents; such as, chlorides, sulphates, sulphides, and carbonates. Also lead in this combination, when injected into the blood stream, will not precipitate the blood proteins. Therefore, I have been able to inject these compounds intravenously in .5 per cent aqueous solution. The results of such injections are recorded in the following paragraphs.

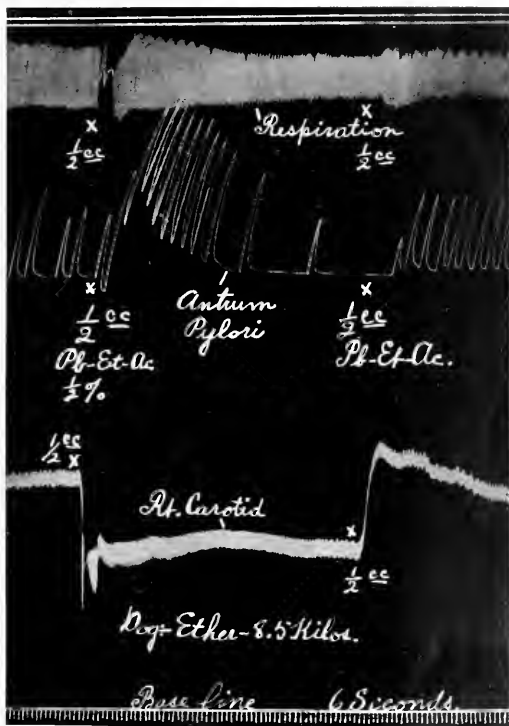


Fig. 1.

#### EXPERIMENTAL

Fig. 1 shows respiration, pyloric contractions, and blood pressure as recorded in a dog of 8.5 kilos under ether anesthesia. At the beginning (left hand side of the record) there was injected .5 c.c. of a .5 per cent water solution of the triethyl lead acetate. It will be noted that the respiration is completely

stopped. There is a marked increase in tone and activity of the pylorus, and a prompt, pronounced and prolonged fall in blood pressure.

The dose, .5 c.c. of .5 per cent triethyl lead acetate, figured in grams of substance injected, is found to be .0025 grams, or about .0015 grams of lead.

The quantity of lead necessary to produce poisoning is not definitely known. Bronardel<sup>14</sup> states that poisoning may result from 1 mg. of lead per day while others give higher figures. Gaertner<sup>15</sup> claims that symptoms will occur in man only after several months with the daily ingestion of from 4 to

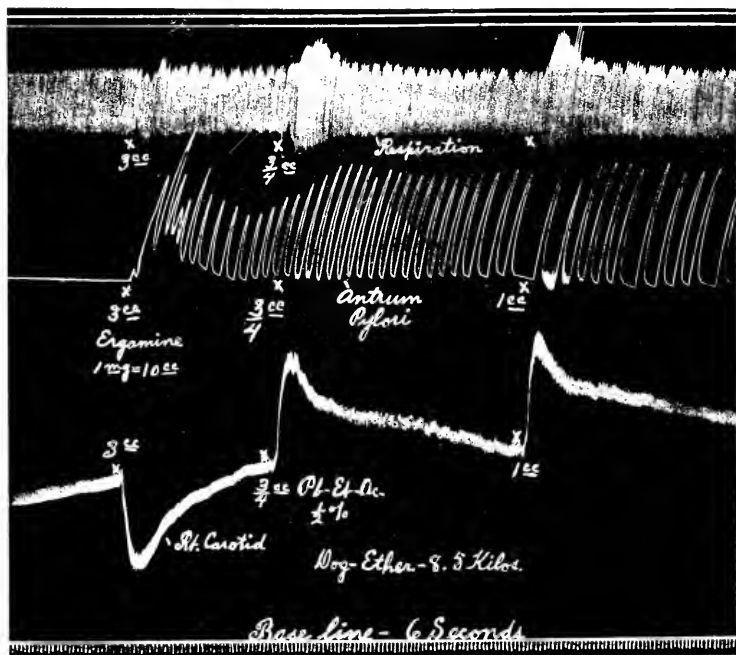


Fig. —

7 mg. According to Flügge and Heffter<sup>16</sup> such symptoms will occur in three or four weeks with the daily ingestion of 60 mg.

The objective symptoms occurring after the first injection of the compound have been recorded by Harnack<sup>17</sup> and are the observations made on an unanesthetized animal. However, they are, for the most part, quite similar to the present observations made on anesthetized animals.

According to Harnack, "the first symptoms following immediately after the injection are doubtless due to the action of the intact compound. The animal yelps loudly and falls in a faint, as if partially narcotized, during which the respiration is completely stopped. If one introduces artificial respira-

tion, the animal will, in the course of a few minutes, breathe and awake from the faint. It is still somewhat dazed and weak and there appears to be psychic irritation, hallucinations, loss of higher functions of the brain, etc. There is also pronounced salivation with a flowing from the nose and a condition of nausea is present; however, without any vomiting."

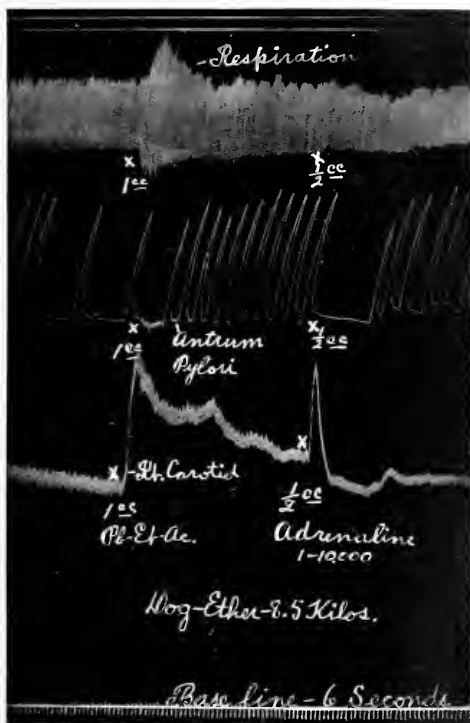


Fig. 3.

It will further be observed in Fig. 1 that the *second injection* of the same amount of the compound is followed by an entirely different result. The blood pressure, instead of falling, rises and remains quite as high as it originally was. Intestinal activity is again somewhat increased and respiration, although poorly shown here, is stimulated instead of being stopped. This striking reversal of conditions apparently completely escaped the attention of Harnack.

Fig. 2 is another record made on the same animal as used in Fig. 1. It shows the result of: first, the injection of 3 c.c. of ergamine solution (1 mg. per 10 c.c.) giving a normal effect for ergamine. The second injection is

$\frac{3}{4}$  c.c. of the .5 per cent triethyl lead acetate solution. The effect on respiration is better shown than in the previous tracing. The third injection of 1 c.c. of the drug, which is the fourth injection of the compound, gives results similar to the previous injection.

Fig. 3 is the third tracing taken from the same animal and shows the result of injecting another cubic centimeter of the triethyl lead acetate solution,

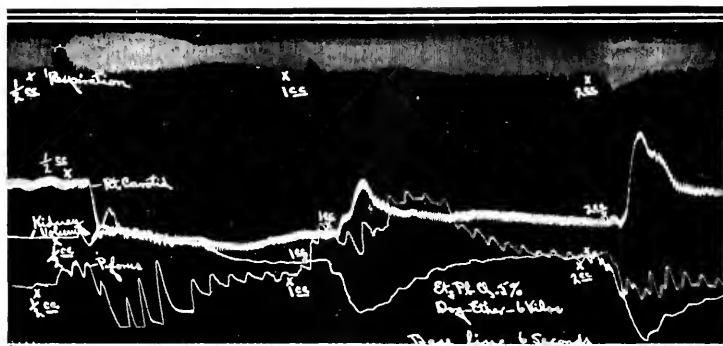


Fig. 4.

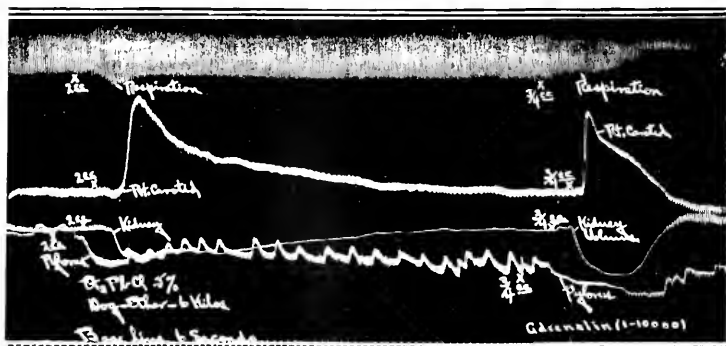


Fig. 5.

the respiration being stimulated, pyloric activity increased, and blood pressure markedly increased. The result of injecting  $\frac{1}{2}$  c.c. of adrenalin (1-10,000) is also recorded and furnishes a comparison with the lead compound as well as a check on the technique.

Fig. 4 shows respiration, blood pressure, kidney volume, and pyloric contractions taken on a dog of 6 kilos under ether anesthesia. The first injection of .5 c.c. of .5 per cent triethyl lead chloride gave similar results to those obtained from the acetate salt, the respiration being stopped, blood pressure fal-

ling, and pyloric toms and activity increased. In addition, it will be seen that the kidney volume, although but slightly altered, did show the heart beat much more plainly than before the injection. The second injection of 1 c.c. of the drug showed practically the same results as those obtained from the second injection of the acetate, with the additional fact that the kidney is constricted. The third injection of 2 c.c. of the chloride shows the stimulation of the respiration and the marked rise in blood pressure, also a decrease in kidney volume.

Fig. 5 is a second record made from the same animal and shows the result

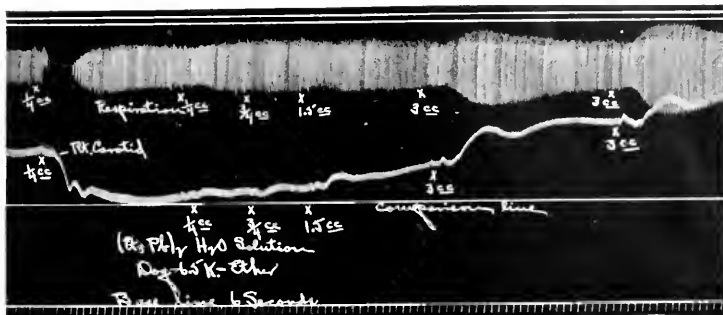


Fig. 6.

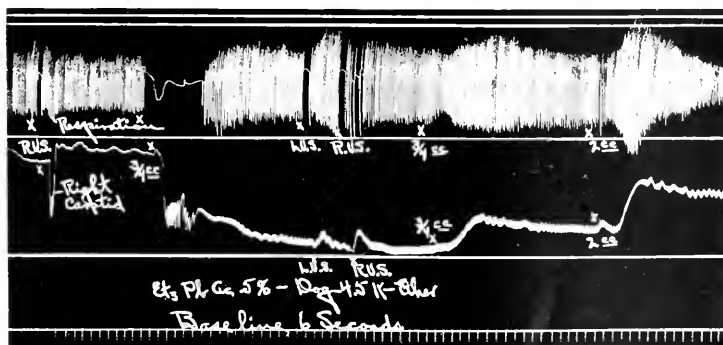


Fig. 7.

of the fourth injection of the chloride, and at the right hand side of the record is shown the result of injecting  $\frac{3}{4}$  c.c. of 1-10,000 adrenaline.

Fig. 6 is a record made on an animal of 6.5 kilos under ether anesthesia, and shows the respiration and blood pressure with the result of injecting an aqueous solution of the triethyl lead. The water solubility of this compound has not been determined exactly; however, it appears to be but slightly soluble. The first injection of  $\frac{1}{4}$  c.c. of such an aqueous solution gave a very similar result to those obtained from the use of the salts previously discussed.

With additional injections, the reversal of the first results did not occur so promptly, but this might be due to the size of the doses administered, and not to any marked difference in their action.

The characteristic fall in blood pressure following the first injection and the rise following the second injection, would suggest the possibility that the vagal action upon the heart was involved in producing the first fall and that

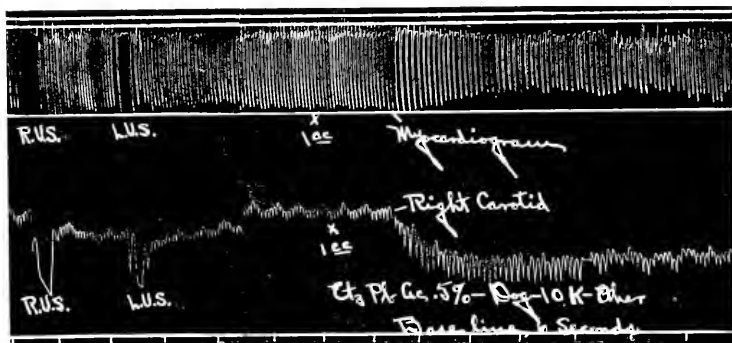


Fig. 6

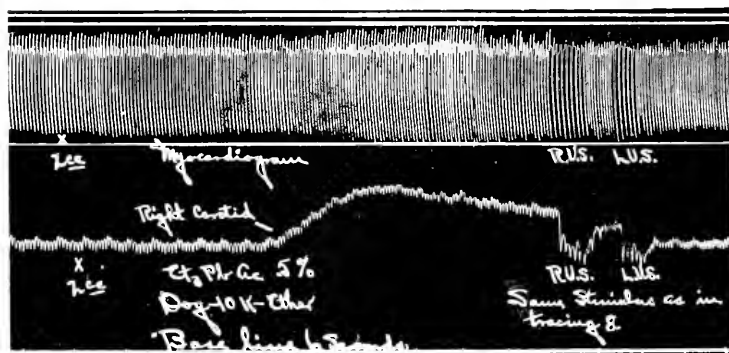


Fig. 7

such action was not present at the time of the second injection. Fig. 7 is a record which apparently supports this suggestion. It shows the respiration and blood pressure in a small dog of 4.5 kilos under ether anesthesia. At the left side of the record at the point marked R.U.S., the right vagus was stimulated in the neck and the characteristic results of such stimulation are recorded. There was injected 3<sub>4</sub> c.c. of triethyl lead acetate solution, and after about two minutes the left vagus was stimulated, causing a stoppage of respiration and an asphyxial rise in blood pressure. However, the vagal stimulation



showed no direct action on the heart. Stimulation of the right vagus gave similar results. These results would lead one to believe that the first action of the drug on blood pressure is due to vagal stimulation followed by paralysis.

Harnack, in discussing the action of the triethyl lead acetate on the rabbit, says:<sup>15</sup> "Also the heart muscle and the muscles of respiration take part in the paralysis, as is indicated by the weak pulse, the slow superficial respiration, the lowering of temperature of the peripheral parts and the weak filling of the blood vessels in the periphery of the body." Desiring to determine whether or not such a weakening of the heart muscle was responsible for the lowered blood pressure in the *dog*, I made myocardiograph records such as is shown in Fig. 8 which is a record of the blood pressure and direct heart muscle action.

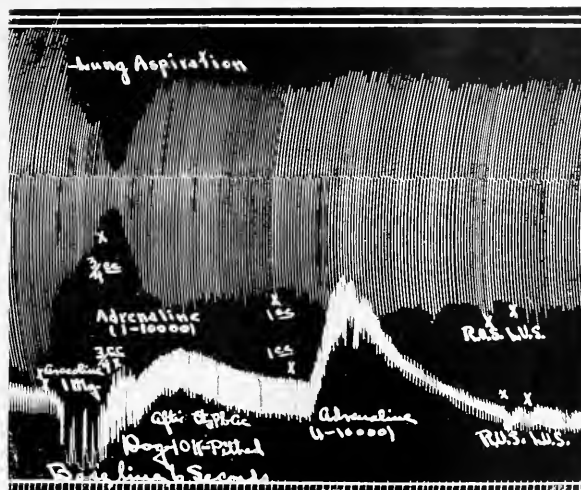


Fig. 10.

At the left side of the record, the right vagus was stimulated. This produced a slowing of the heart with a fall in blood pressure. Stimulation of the left vagus gave similar results. After a normal blood pressure was again established 1 c.c. of the .5 per cent triethyl lead acetate was injected. It will be noted that about 6 or 8 seconds later there occurred the characteristic fall in blood pressure, which was accompanied by a slowing and a weakening of the heart beat. Fig. 9 shows the result (taken on the same animal as was used in record 8) of injecting 2 c.c. of the solution. In about 24 seconds the blood pressure rose and the heart beat was more rapid and stronger. Stimulation of the right and left vagus, with the same strength stimulus as used in record 8, showed the vagus was still active, but not so strongly as in Fig. 8.

At this phase of the work it was thought important to determine whether the innervation of the bronchial musculature was also affected similarly to

that of the heart. Fig. 10 shows the result of such observations. This animal was pithed and, after receiving 20 c.c. of triethyl lead acetate solution, 2 c.c. at a time, the record (Fig. 10) was made. The first injection at the left was 1 mg. of arecoline. The normal action of arecoline on the lung volume and blood pressure is observed. After constriction of the lungs by the arecoline,  $\frac{3}{4}$  c.c. of adrenaline 1-10,000 was given with the result that adrenaline dilated the lungs in the normal manner. Another c.c. of adrenaline was again injected, which produced a further dilatation. It will be noted that vagal stimulation (R. V. S. and L. V. S.) had no direct action on the heart. It would appear from this record that the drug does not paralyze the endings of the postganglionic fibers of the vagi, or of the sympathetics in the lungs.

In attempting to determine whether or not the sympathetic ganglia are acted upon by the drug, a series of experiments were carried out, using lobelin.

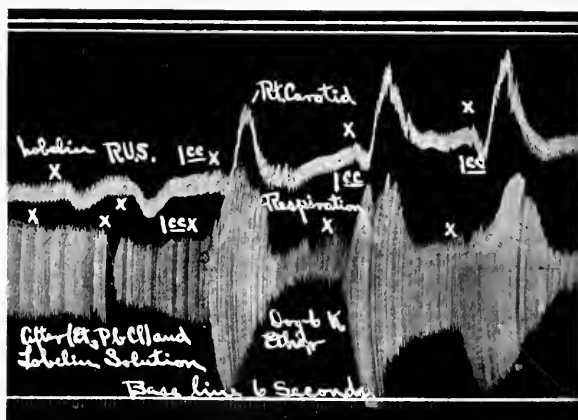


Fig. 11.

Lobelin is a drug which first stimulates and later paralyzes the sympathetic ganglia,<sup>19</sup> an action quite similar to that of nicotine. Fig. 11 shows a record taken on an animal which had previously received three or four injections of triethyl lead chloride, and had given a normal response to each. A small amount of lobelin solution was then injected and the usual reaction, due to stimulation of the sympathetic ganglia, was observed. The record shows the result of a second injection of lobelin, which by the lack of response, indicates that the ganglia are paralyzed. As a further check, the right vagus was stimulated with the result shown. It will be observed that, after the administration of lobelin, the results of administering triethyl lead are very similar to those obtained from a normal animal. In answer to the question as to whether or not a primary fall in blood pressure, following the first injection of triethyl lead salts is obtained after lobelin, we may refer to Fig. 12. This tracing shows the respiration and blood pressure as recorded in a dog

of 8.5 kilos under ether anesthesia. The blood pressure changes in this particular animal, following the injection of lobelin and triethyl lead solution, are not very pronounced. However, they are completely typical, and the record serves to show that the triethyl lead acts in its usual manner after the injection of lobelin.

Harnaek, in discussing the fall in blood pressure, produced in rabbits, maintains that the vessel walls are not acted upon, and that the vasomotor center is not affected, but that the whole action is due to the weakened condition of the heart. As evidence he submits the following: "To decide this question, the following course of research was carried out: a rabbit which showed the action of triethyl lead decidedly, was, while in such condition, examined to note the state of the ear vessels. Such animals, if then subjected to chloro-

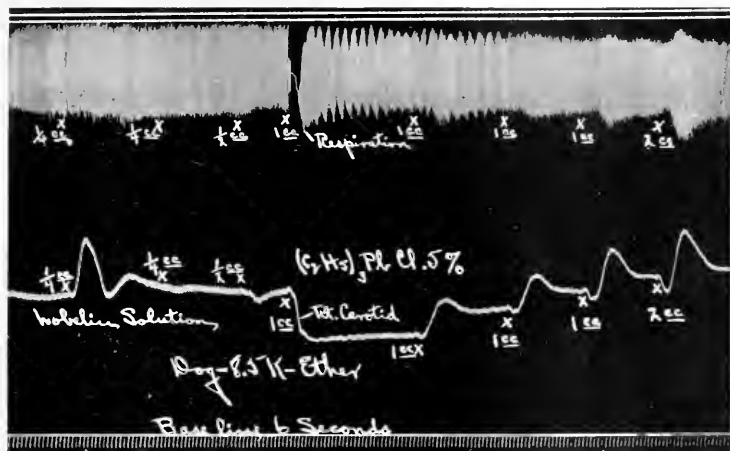


Fig. 12.

form anesthesia, showed a widening and filling of the ear vessels, which were previously narrow and weakly injected. This shows that the vessel muscles are not directly stimulated by the lead." In order to show that it is not the vasomotor center which is affected, he carried out the following experiment. In a rabbit (1570 grams) he cut the sympathetic nerve on the right side of the neck and, after the vessels in the right ear dilated and increased in temperature, he injected .040 grams of triethyl lead acetate subcutaneously. The following morning there was only a slight beginning of paralysis. Therefore, another small quantity of the salt was given (amount not stated). Very soon the paralysis became greater, the heart beat weak, and the animal cool. The vessels in both ears were bloodless, and those in the right remained wider than in the left. Chloroform was then given, and the left ear vessels dilated. The animal died during the anesthesia from "heart paralysis." There were therefore no grounds, he decided, for supposing that the lead stimulated the vaso-



tion of the drug produced any volume change in the spleen or kidney. Fig. 13 is a record taken in the course of such work and shows respiration, spleen volume and blood pressure. The first two injections, shown on the record, are those of lobelin sulphate solution and are the second and third injections. It will be noted that the lobelin had no noticeable action, which means that the first injection (not shown in the tracing) was sufficient to paralyze the sympathetic ganglia. The injection of 1 c.c. of the .5 per cent triethyl lead chloride gave complete stoppage of respiration, an initial increase in spleen volume followed immediately by a decrease, and the blood pressure was lowered. The second injection of the drug stimulated respiration, decreased spleen volume, and increased blood pressure.

Fig. 14 shows respiration, spleen volume, and blood pressure. The injection of  $1\frac{1}{2}$  c.c. of 1:10,000 adrenaline solution gave the characteristic action

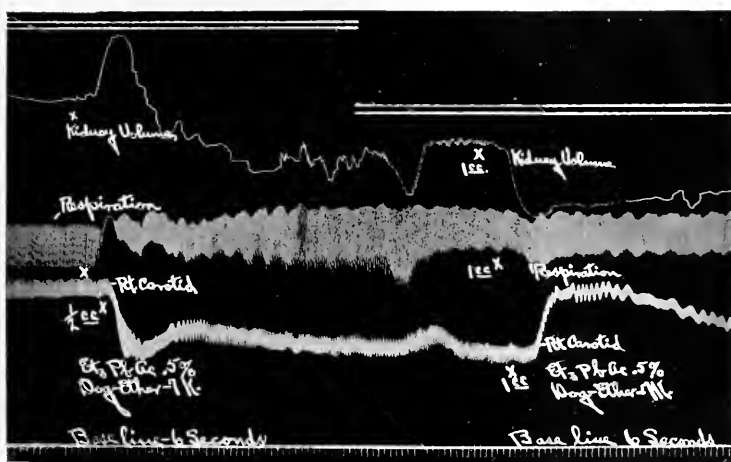


Fig. 15.

on the spleen volume and some increase in blood pressure. The injection of 1 c.c. of a .5 per cent solution of the lead salt gave stoppage of respiration, an increase in spleen volume followed immediately by a decrease, and the blood pressure fell as usual. The principal thing of interest in the second injection of lead (2 c.c.) is the marked contraction of the spleen *without the preliminary dilatation*.

Fig. 15 shows kidney volume, respiration and blood pressure. It will be noted that with the first injection the kidney volume increased. This increase in volume is followed by a not very marked, but prolonged decrease. The second injection of the drug gave only the decrease in kidney volume.

Fig. 16 is presented to show the initial increase in kidney volume, followed immediately by a pronounced decrease in volume. It will also be noted that, with this first injection of the drug, there is a marked increase in tonus activity

of the pylorus. In this connection, reference to Fig. 1 will show that intestinal activity was there also markedly increased.

In order to determine whether or not the vagus endings are the seat of action of the lead salts, the triethyl lead acetate was administered after atro-

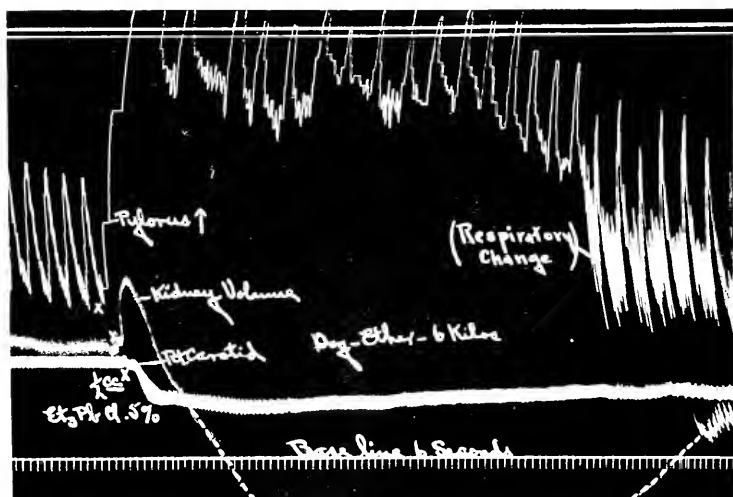
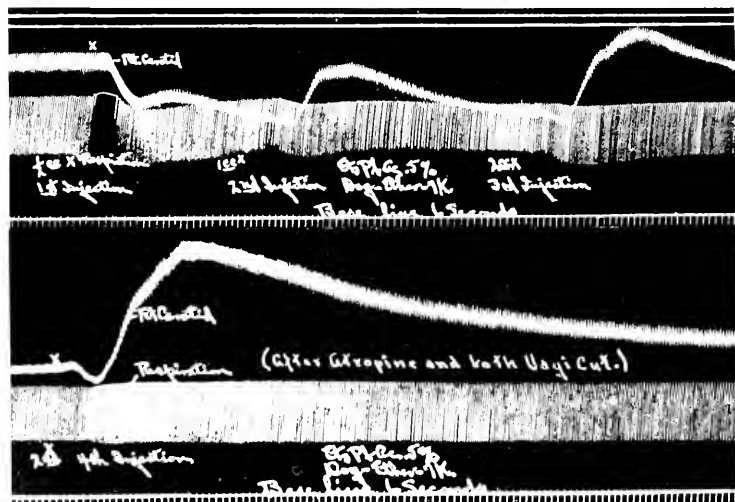


Fig. 16.



pine, and also after atropine and section of both vagi. Fig. 17 is a record of blood pressure and respiration recorded in an animal after the administration of atropine and section of both vagi. It will be noted that the first injection (*upper half of tracing*) produced a cessation of respiration and a fall in blood pressure, somewhat similar to the effect observed on animals with intact vagi; also the subsequent injections of the triethyl salt gave results similar to those obtained from normal animals. It is of interest to note the relative height and duration of the second and fourth injections.

Fig. 18 shows lung volume and blood pressure as recorded in a pithed dog of 6.5 kilos. Following the injection of .5 c.c. of .5 per cent triethyl lead chloride solution, the lungs show a slight but insignificant increase in volume. The blood pressure was gradually somewhat lowered and remained at a constant level regardless of the later injections of 1, 2 and 4 c.c. of the

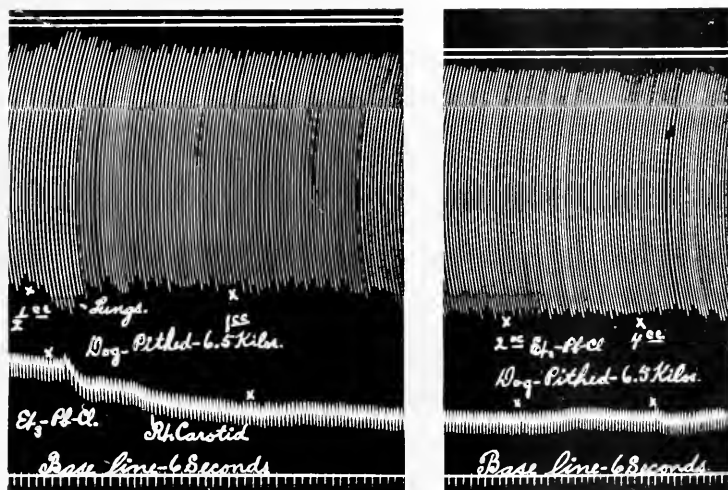


Fig. 18.

drug. It is evident that the drug has but little apparent action on a pithed animal. There are those who maintain that the dyspnea accompanying lead colic, is due to a spasm of the bronchial muscles.<sup>31</sup> However, it will be observed that lead, in such a form as here injected, does not cause a constriction of the bronchioles, but, if anything, a slight dilatation.

In order to further localize the seat of action on the central nervous system, the cord was sectioned below the origin of the phrenic nerves and the drug was then injected. Fig. 19 shows a record of such an experiment. The animal was breathing normally, as is shown by the record, and it will be observed that the respiration was greatly influenced by the drug, but that blood pressure shows only slight changes; the initial rise is possibly due to the action of the diaphragm. The injections following the first showed no significant ac-





The most interesting part of the record is that the first injection produced a slight rise in blood pressure, and not a fall. The second injection of 1 c.c. did not alter the blood pressure, but did affect the respiration, making it necessary to introduce artificial respiration.

Desiring to determine whether the activity of the compound is due to the particular salt, or to the lead in the molecule, or to the special arrangement

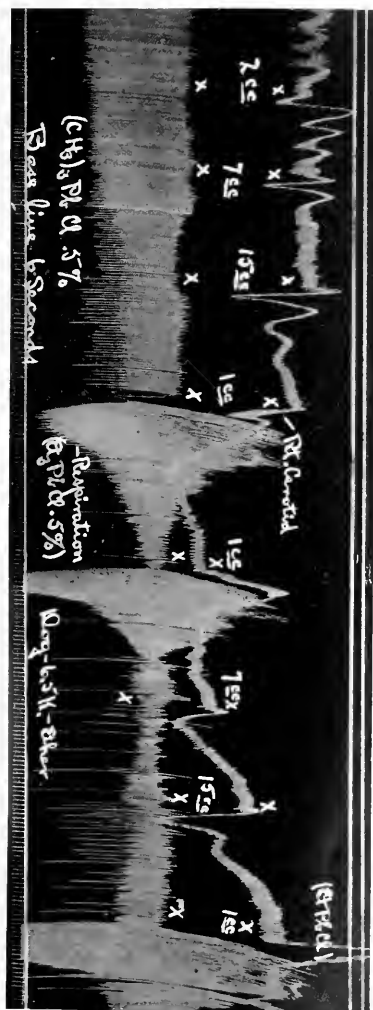


Fig. 21.

of the ethyl radicals in the molecule, I extended the work to include the preparation and use of the trimethyl lead, trimethyl lead chloride, and triethyl antimony nitrate. That the action is not due to the particular salt of lead triethyl is evidenced in the work already presented.

The preparation of trimethyl lead is described by Cahours;<sup>21</sup> however, I prepared it in much the same way as the ethyl compounds, and for the chloride I obtained a preparation of white, thin, crystalline plates, having an odor not unlike the methyl amines. I did not run an analysis on the product, and for that reason, am not certain as to its absolute purity; however, as a check on the work, I used an aqueous solution of the oil, which is  $(\text{CH}_3)_3\text{Pb}-\text{Pb}(\text{CH}_3)_3$  and later a 50 per cent alcoholic solution of the compound. The oil must necessarily be of a rather high degree of purity.

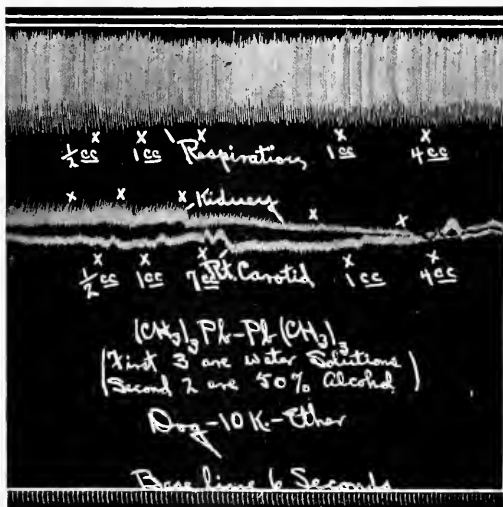


Fig. 22.

Fig. 21 is a record of blood pressure and respiration taken on a dog under ether anesthesia. The first injection is that of 2 e.e. of .5 per cent *trimethyl lead chloride*. There is no apparent change in the respiration, and the blood pressure shows only a slight fall immediately followed by a greater rise. The next two injections of 7 and 15 e.e. correspond quite closely to the first injection. The injection of 1 e.e. of triethyl lead chloride solution at this point gave the characteristic fall in blood pressure and a stoppage of respiration, followed after a brief interval by a pronounced stimulation. The second injection of 1 e.e. of triethyl lead salt acted in the usual manner. The next two injections of 7 and 15 e.e. are of the methyl compound and show nothing very striking. The last injection of the ethyl compound gave a very pronounced result.

As a further check, the aqueous solution of the trimethyl lead was used. It will be noted in Fig. 22 that such a solution did not show any action on respiration or blood pressure. The kidney volume did show that filling of the kidney was not so good as time went on; this may be due to the action of the compound. Not knowing the solubility of the compound in water, a saturated solution in 50 per cent alcohol was used, which shows no pronounced changes. Fig. 23 is another record taken on the same animal as used in Fig. 22. It will be noted that a water solution of the triethyl lead was very active. The third injection of 3 c.c. proved to be a fatal dose.

These records imply that either one or the other of two things is responsible for the activity of triethyl lead compounds; either that the lead radical in that particular combination is responsible; or that the activity is due to

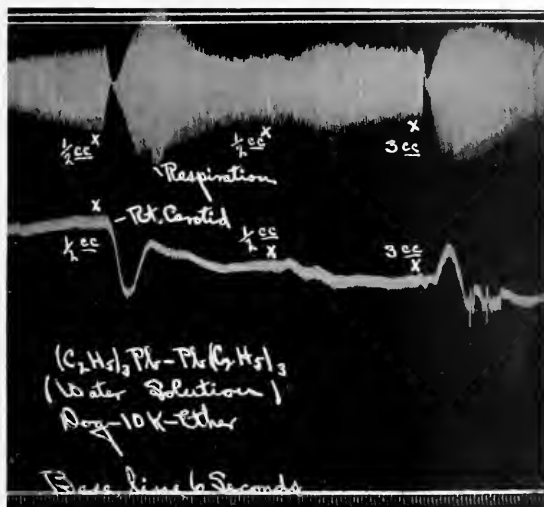


Fig. 23.

the peculiar combination of the ethyl radicals with the lead. Apparently trimethyl lead does not act in the body as the triethyl lead compounds do. This point is not so startling when one considers that there are many substances which differ chemically much less than triethyl lead and trimethyl lead differ (for example, some of the sugars), but which, when taken into the body, vary greatly in their assimilability by the tissues (as occurs with levulose and dextrose).

To determine whether the activity of the triethyl compounds is due mainly to the particular grouping of the ethyl radicals, I have prepared and used *triethyl antimony nitrate*. The triethyl stibine is obtained by the action of ethyl iodide on an alloy of antimony and sodium in a manner similar to that used in the preparation of the triethyl lead.<sup>22</sup> It is a colorless liquid having an

odor of onions, and on coming in contact with the air, oxidizes instantly and burns with a white flame. Triethyl stibine oxide is obtained by shaking an alcoholic solution of the triethyl stibine with finely divided mercuric oxide. Mercury is set free, and the oxide of the radical is produced.

The nitrate can be prepared by: First, the action of nitric acid on the triethyl stibinic oxide; or, second, the action of dilute nitric acid on the triethyl stibine. The nitrate crystallizes out in well defined, rhomboidal crystals with a melting point of  $62.5^{\circ}\text{C}$ . It is very soluble in water and has a bitter taste.

Fig. 24 shows respiration and blood pressure as recorded in an animal of 10 kilos under ether. Triethyl antimony nitrate was injected in a 1 per cent water solution. It will be noted that the results of the injection in no way correspond to those obtained through the use of the triethyl lead salts. It is

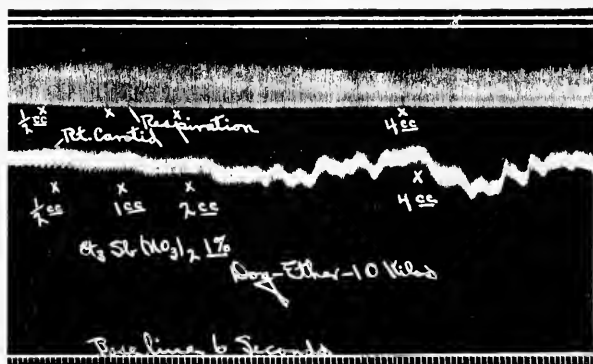


Fig. 24.

of interest that, after this tracing was made, the lead salt was injected and the characteristic results obtained. From this, it appears that the ethyl radical is not chiefly responsible for the activity of the triethyl lead compounds.

#### EXCRETION

Lead is excreted mainly by the feces (small and large intestine and bile) and to a minor degree in the urine; also traces appear in the saliva, sweat and milk.<sup>23</sup> After ingestion all is excreted in organic combination, except the unabsorbed fraction in the feces.<sup>24</sup>

Dauwe<sup>25</sup> states that lead injected intravenously disappears rapidly from the blood, the greater part disappearing in less than two minutes. Kobart<sup>26</sup> claims that the main deposition of lead in the body occurs in the kidneys, the bones, liver and other glands, a minor amount in the brain, striped and smooth muscle, and blood.

In attempting to determine the mode and rate of excretion of the triethyl lead compounds, Harnaek failed,<sup>27</sup> after numerous attempts, to show the presence of any undecomposed triethyl lead in the urine of animals poisoned with the compound. He maintained that, if such a radical should be present,

it would be easy to isolate it and to demonstrate its presence. He presents the following data obtained from large samples of urine, collected for long periods of time:

Three and one-half liters of urine, collected from a rabbit which had received during the period (45 days) a total of 0.3 grams triethyl lead acetate administered subcutaneously, gave a yield of .0077 grams of lead. There was therefore about 4 per cent of the lead excreted in the urine. The feces were not analyzed.

In the present work, I have, on several occasions, tested for the presence of lead in the samples of urine obtained from the animal at the end of the experiment. These animals would often receive a total injection of .125 grams during the experiment, and occasionally as much as .250 grams have been given, the duration of the experiment often being two hours or more. However, I have not, in any case, been able to detect the lead in the urine, either by applying the test to the urine directly, or by evaporating it to dryness in the presence of concentrated nitric acid and testing for the lead salt. However, I do not maintain from these findings that lead is not to a certain degree excreted in the urine.

#### DISCUSSION

With the foregoing experimental data at hand, it is very interesting to review the conclusions formulated by Harnack. He considers that interpretation of his results justify the following conclusions,<sup>28</sup> translated somewhat freely:

(1) That lead affects the substance of all cross striated muscle; not that it primarily makes each contraction impossible, but that it causes the active muscle to become quickly exhausted; also the muscle finally loses its irritability and dies; yet the lead does not interfere with rigor mortis. This action of lead is more pronounced in the frog and rabbit than in other animals.

(2) That lead affects certain central motor nerve mechanisms; the action consists of a stimulation, from which originate peculiar ataxic movements, also a continuous trembling and twitching, and finally convulsions; yet, consciousness remains and sensibility is not lost. The seat of action is probably the mid-brain or cerebellum. The action is especially marked in the dog, but is also noted in cats and pigeons.

(3) That lead irritates certain nerve structures, located in the intestinal wall, which control the intestinal movements. This stimulation therefore causes general contraction and increased peristalsis of the intestines, producing colic and causing an increased irritability of the whole belly, also diarrhea. The action is clearly outspoken in all mammals which were studied. An action on the smooth muscle of the intestine, of the blood vessels, etc., is not demonstrable. Respiration and circulation are not directly influenced, except that in animals in which the muscle paralysis is very prominent, then also the heart and respiratory muscles take part in the paralysis.

Although the records which I have presented do, to a certain degree, confirm some of Harnack's observations, there are, however, many points on which we differ widely. One of the most striking differences deals with the questions of respiration and circulation. Harnack was of the opinion that these were not primarily affected; however, there seems to be no doubt that, as

shown by my experiments, both respiration and circulation are greatly affected by the administration of the triethyl lead compounds. To determine the manner in which respiration and circulation are affected has been the object of a great deal of the present work.

It is generally assumed that chronic lead poisoning is accompanied clinically by a rise in blood pressure and vasoconstriction. Riegel and Frank<sup>20</sup> stated that, during the attacks of colic, the blood pressure was increased, and it has therefore been suggested that the hypertension may be due to the violent contraction of the intestines which produce mechanical displacement of blood, or to the pain reflexes. The pallor of the skin has been considered an indication of vasoconstriction. Quellien<sup>21</sup> believes that even in acute poisoning the blood pressure is raised.

That the blood pressure changes as produced with lead triethyl salts are not due to the increased activity of the intestines with the forcing out of the blood from the intestinal area is clearly shown in Fig. 1 and again in Fig. 16, in which it will be observed that, regardless of the violent intestinal activity produced by the injection of the first dose, the blood pressure falls and remains low until the second injection of the compound. At this time peristalsis is not any more active, if even as vigorous, as following the first injection; yet the blood pressure is strikingly increased.

That the blood pressure changes are not wholly due to muscular activity resulting from central nervous stimulation is evident, for there is present as much or more, muscular activity following the first injection as there is after the second; yet, a fall in pressure is produced by the first injection instead of a rise. Also, if an animal is deeply under anesthesia so that no muscular activity is observed, the fall and rise are produced in the usual order.

That the cessation of respiration in the first case and the stimulation in the second are not directly responsible for the blood pressure change is especially well shown in Fig. 17 and others, where it will be observed that the intensity of the blood pressure change does not, in any degree, correspond to the respiratory changes.

One factor which appears to be quite active in producing alterations in the blood pressure is the variation produced in the rate and volume output of the heart following the first and subsequent injections. This point is shown in Figs. 8 and 9. After the first injection of the compound, the heart beats more slowly and the amplitude of the beat is considerably less. This action appears to be partly due to stimulation of the vagus. But Fig. 9 shows that, following the second injection, the heart rate and amplitude are considerably increased. It appears that the cardiac vagal action present at first has disappeared, and it was found in several animals, the tracing from one of which is shown in Fig. 7, that stimulation of the vagus with the electric current was either without action, or the action was much less, after the administration of the drug. From the records presented one is likely to doubt this proposed explanation of vagal stimulation and paralysis, since Fig. 17 was obtained after atropine and Fig. 13 after lobelin, and both show changes somewhat similar to those obtained from animals with intact vagi. However, a more careful consideration will show that the fall in blood pressure in both

of these cases was not a sudden fall such as results from electrical stimulation of the vagus, or, as that observed in Figs. 1 and 7.

The sudden dilatation of the spleen and kidney, following the first injection might also play some part in the fall of blood pressure, while the constriction of these organs evidently plays an important part in the rise following the subsequent injections.

Animals, injected with the triethyl lead compounds, unless deeply under anesthesia, showed a marked stimulation of the higher centers, at times the stimulation being so intense that the animal would wink its eyes and apparently regain consciousness, although it was completely under the anesthetic just at the time of injection. These results and the violent action on the respiration prove that the drug acts on the higher centers and the medulla.

It is very interesting that triethyl lead and its compounds should be so active in stimulating the higher centers and the medulla, for it would be expected that, if the ethyl radicals exerted any action, this would be in the nature of depression, for such is the property of most of the ethyl compounds; for example, ether, ethyl bromide, etc. And again, it would scarcely be expected from the general character of chronic lead intoxications that the metal possessed strongly stimulating properties; therefore, it is rather surprising to find the compound so extremely active.

This violent stimulation of the medulla and basal ganglia is, beyond all doubt, responsible for most of the symptoms resulting from the administration of lead in such a combination. It will be observed that the drug has practically no action on blood pressure when administered to a pithed animal. Also in a dog with the cord sectioned at the seventh cervical vertebra, the result is practically the same, while after section of the cord and both vagi, the blood pressure shows a slight, but insignificant rise.

A pithed animal (brain and medulla), or an animal in which the cord has been sectioned at the seventh cervical vertebra shows no signs of convulsions. This means that the medulla and possibly the higher centers are responsible for the convulsions.

The violent stimulation of the respiratory center in the medulla explains the changes produced in respiration. It seems possible that the stoppage of respiration following the first injection is due to extreme stimulation of the center, (or, of some higher controlling and inhibiting mechanism,) and that the increased respiration following subsequent injections is the result of direct stimulation of the center itself after its normal hypersusceptibility to the compound has been slightly depressed by the first injection of the substance.

The kidney and spleen volume changes also strongly suggest stimulation of the vasomotor centers. The primary dilatation is apparently of central origin. But I have suspected many times that, in addition to this action, there might be some peripheral mechanism concerned, possibly in the nature of a brief primary stimulation of vasodilator nerve endings. The marked shrinkage in volume of the kidney and spleen which comes on secondarily is certainly due mainly, and perhaps entirely, to stimulation of the medullary vasoconstrictor center. It is exceedingly probable that this same action is the

cause of the prolonged hypertension which characterizes chronic lead poisoning.

There is also a very strong probability that the increased intestinal activity, which gives rise to colic and other intestinal disturbances, including constipation, following the ingestion of lead is of central origin. And the slow pulse of chronic lead intoxication may very well be due partly, and perhaps mainly, to stimulation of the medullary inhibitory center.

While in the early part of this work I was inclined to suspect that the sympathetic ganglia might be the seat of action of the metal in the production of various phenomena, especially in regard to the effects on intestinal peristalsis; yet, in the long run I have been unable to find any positive evidence that the ganglia are directly affected. In Fig. 19, for example, it was found that repeated injections of triethyl lead chloride produced no rise whatever in blood pressure after section of the spinal cord at the level of the seventh cervical vertebra. If the vasoconstrictor ganglia had been stimulated in this case, as would have occurred under nicotine the blood pressure should have risen as in the normal intact animal.

#### CONCLUSIONS

(1) Triethyl lead and salts of triethyl lead are extremely active in stimulating the central nervous system. The stimulation is confined mainly to the medulla and higher centers including the pons, midbrain, and perhaps certain portions of the cerebellum and cerebrum.

(2) The injection of from .0025 to .0050 grams of the salt is sufficient to produce the characteristic action in a medium sized dog, the most conspicuous objective symptom being the production of convulsions. In character, these convulsions correspond to those produced by cyanides and picrotoxin, but, in degree, approximately to those of strychnine.

(3) There is an extreme fall in blood pressure following the first injection of the compound, but a marked and prolonged rise in blood pressure following all subsequent injections. The fall in blood pressure is due to at least two, and probably three factors: (1) Stimulation of the inhibitory vagus center for the heart, (2) sudden dilatation of the vessels of certain visceral organs including the kidney and spleen, and (3) a direct depressant action on the heart. The rise in blood pressure following the second or subsequent injections is due to (1) constriction of the vessels of the kidney, spleen and possibly other organs, (2) stimulation (in the medulla) of sympathetic nerves to the heart, (3) general systemic convulsions in case these are present. But the secondary rise in blood pressure occurs independently of the existence or absence of general convulsions, although these, if present, increase the extent of the rise.

(4) Respiration is stopped by the first injection and greatly increased by subsequent injections. The primary cessation appears to be due to excessive stimulation of the center itself, or, of some higher inhibiting nervous mechanism, probably located in the pons or midbrain. Acceleration and deepening of the respiration, which accompany later injections, are doubtless the result of direct stimulation of the center itself after its hypersusceptibility to the drug has been somewhat decreased by the first injection.

(5) Following the primary injection of lead triethyl acetate, the kidney



and spleen volumes undergo an increase, followed immediately by a decrease, but only the decrease in volume is produced by subsequent injections. The preliminary increase in volume is probably due to stimulation of the vasodilator center in the medulla, while the shrinkage in volume of these organs following the second or subsequent injections is due to a direct stimulation of the vasoconstrictor center. (The preliminary dilatation may, of course, be explained as the result of inhibitory stimulation affecting indirectly the vasoconstrictor center.)

(6) The dyspnea accompanying injections of the drug is not due to spasm of the bronchial muscles, but to a direct action on the respiratory center.

(7) Probably the increased intestinal activity following intravenous administration of the compound is the result of medullary stimulation which thus indirectly increases the activity of the vagus nerves. This may also account for the slow pulse, the increased peristalsis and colic, and the dyspneic, or asthmatic symptoms, of chronic lead poisoning.

(8) The salts of triethyl lead furnish excellent examples of compounds which contain three ethyl groups (which are ordinarily considered to act as depressants on the central nervous system), but which, either owing to the peculiar relations existing in the composition of the molecule as a whole, or else by the specific action of the lead contained in the molecule, act as strong central nervous stimulants, especially in the medulla, pons and midbrain, and perhaps even in the motor areas of the cerebrum, or in the cerebellum.

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- <sup>31</sup>Sollmann: *Loc. cit.*, p. 803.

## STUDIES ON THE RESISTANCE OF THE RED BLOOD CELLS\*

### I. RESISTANCE OF THE RED BLOOD CELLS IN HEALTH TO THE HEMOLYTIC ACTION OF SAPOTOXIN

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THE subject of hemolysis has received much attention both from the experimental and clinical aspect. Early workers confined their attention to the degree of resistance offered by the erythrocytes to anisotonic saline solutions. Since then all types of hemolytic agents have been studied, among which have been animal and plant poisons. Early students sought a physical basis for the processes of hemolysis. Soon, physical laws were found inadequate to explain certain phenomena, and the subject was investigated anew from a chemical standpoint.

In brief the results of the two types of hemolytic action—osmotic tension and hemolysins—are as follows: Rather wide physiologic variations in the isotonic tension of the blood normally occurs. The venous blood usually shows a slightly higher tension than that of the arterial blood. The resistance remains remarkably constant in health but in certain diseases there are found wide variations. Resistance of the red cells to anisotonic salt solutions is raised by the addition of hydrogen, nitrogen, arsenic, carbon dioxide, carbon monoxide, and acids and diminished by traces of alkalies or oxygen. During the course of typhoid fever, pneumonia, erysipelas, and other acute infections the isotonic tension may be increased. The resistance is also increased in leucemia, secondary anemias, pregnancy, lactation, obstructive jaundice and carcinoma. In cases of elevated blood-pressure, hemolytic icterus, fever, chlorosis, pernicious anemia and cyanosis the resistance of the red cells is lowered.

Many hemolytic agents such as the glucosides, specific hemolysins, and animal poisons have been considered from the clinical aspect. Probably the most thoroughly studied hemolytic agent is that of saponin. The saponins markedly reduce the surface tension of water, also they hold insoluble bodies in suspension and have a peculiar affinity for lecithin which they dissolve. Cholesterol, on the other hand, forms an insoluble chemical compound with many of the glucosides. It appears that the presence of a hemolytic agent and lecithin permits destruction of the cells, while cholesterol deprives such poisons of their toxicity by forming inactive cholesterides with them. The injection of saponin results in a terrific destruction of the red blood cells and the appearance of the hemoglobin in the plasma. Such a condition cannot be considered the result of changes in the hemoglobin, but rather to the destruction of the stroma of the corpuscle thereby releasing the hemoglobin. The

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saponins possess a strong solvent action upon the lecithin of the stroma, hence its reduction or removal leads to disintegration of the corpuscles. Such solvent action occurs more readily when the blood cells are removed from their plasma or serum, or suspended in an isotonic saline solution. In such cases the red cells are removed from the protection of cholesterol which normally acts to form inactive compounds with saponin.

Substances other than the saponins when present in the blood stream or about the red cells in excess bring about destruction of the red corpuscles. The action of snake venom has long been known; more recently it has been shown that oleic acid in excess has the same hemolytic action as that of the saponins.

The present paper deals with the action of a specific hemolytic agent, sapotoxin, on the resistance of normal washed and unwashed red blood cells. This work was carried on preparatory to a study of the resistance of the red blood cells in various diseases. The results of our studies on the resistance of the erythrocytes in disease and the relation of red cell resistance to blood chemistry, especially to cholesterol, will appear in later communications. During the course of this study over two thousand experiments were performed.

#### METHODS

Merek's preparation of sapotoxin was used throughout the series of experiments. The term sapotoxin is employed to designate the more poisonous glucosides, the term saponin being restricted to include less active ones and certain innocuous isomers of sapotoxin which are formed from them by boiling with alkalis. Sapotoxin is a light cream-colored amorphous powder that goes into solution readily; possesses a sweetish, aromatic odor, and froths excessively upon agitation when in solution.

In order to maintain an isotonic solution in which to study the resisting power of the red cells, all sapotoxin dilutions were made up in 0.9 per cent salt solution. A sapotoxin dilution of 1:1,000 was used as a standard or stock solution from which consecutive dilutions of from 1:7,000 to 1:40,000 were made in quantities desired. The solutions were then sterilized and kept on ice while not in use. New solutions were prepared and checked each week.\*

The technique for determining the resistance of the erythrocytes to sapotoxin solutions was as follows: A series of from 4 to 6 small, clean test tubes were labeled and placed in a water bath especially prepared for the work, Fig. 1. The temperature about the tubes was maintained between  $24\frac{1}{2}$  and  $25^{\circ}$  C. One cubic centimeter of the desired solutions was delivered into each of the tubes. An extra tube of a 1:13,000 dilution was used to determine the time necessary for complete hemolysis upon standing at a constant temperature. The patient's finger was then prepared and punctured for free flow of blood. By means of a capillary pipette, Fig. 1, 20 c. mm. of blood was drawn and delivered into each of the test tubes and mixed by gentle shaking, the time being noted in each case. Mechanical injury to the cells was re-

\*Noguchi has shown that saponins do not deteriorate quickly. However, solutions of sapotoxin are prone to develop growths unless proper precautions are made to keep them sterile.

duced as much as possible as Meltzer and Welch have shown that the erythrocytes have varying resistance to shaking, and that the effect of shaking depends upon the rapidity of vibration.<sup>1</sup> Blood was also obtained for a red cell count and a percentage hemoglobin determination. Having obtained the samples they were removed to the laboratory and each tube allowed to stand exactly five minutes from the time it was delivered into the sapotoxin solution. The tubes were then centrifuged for from 30 to 45 seconds under high

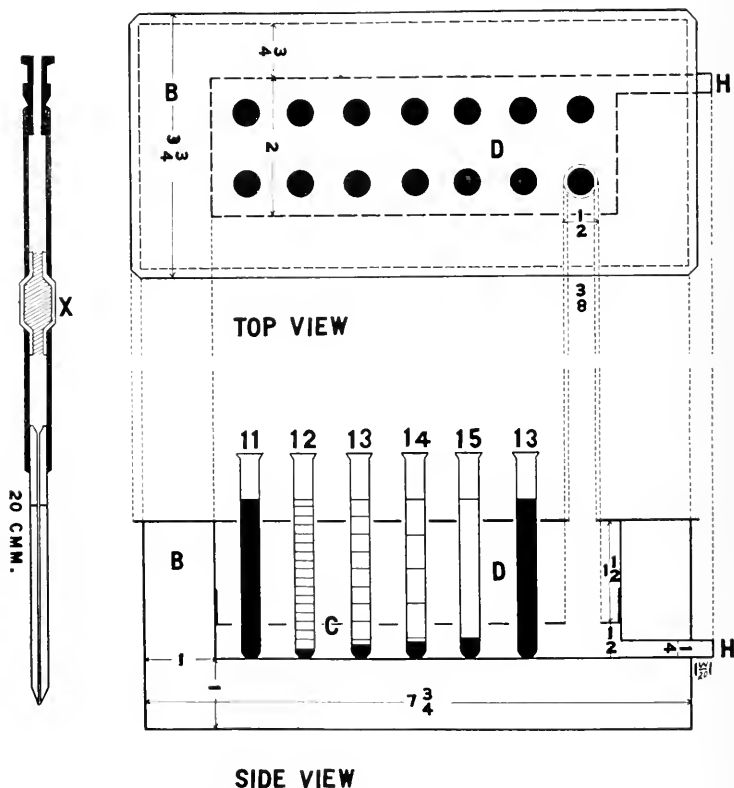


Fig. 1.—Diagram of top and sectional views of test tube container and warmer showing the relation of the double water jacket and test tubes in position. Made of sheet copper to dimensions indicated on drawing. Closed outer jacket *B* has a  $\frac{1}{4}$  inch vent *H*. The inner portion *D* shows holes placed for tubes. A false bottom *C* with perforations corresponding to those of the top but  $\frac{1}{8}$  inch greater in diameter maintains the tubes in a vertical position. The shadings of the tubes represent various degrees of hemolysis in a given sample of blood at the end of five minutes plus centrifugalization. Hemolysis is shown as complete in tube 1:11,000 strength solution; good in 1:12,000; fair in 1:13,000; trace in 1:14,000; absent in 1:15,000. The second 13 tube, 1:13,000 solution, shows complete hemolysis of red cells on standing at constant temperature. The solid black in the bottom of the tubes indicates the relative amounts of unbroken cells thrown down after centrifugalization.

To the right of the picture is shown a modified Sahli's hemoglobinometer pipette. The portion marked "X" is a piece of glass tubing filled with cotton wool. This simple modification insures evenness of drawing and prevents blood from entering the mouth.

speed. An electrical centrifuge was chosen because of the rapidity with which the unbroken cells could be thrown out of solution. The ordinary water centrifuge is entirely undesirable because of the small number of revolutions possible per minute; its slowness adds to the length of time the red cells are exposed to the action of the hemolytic solution. After centrifugalization the tubes were compared with each other against white light in order to determine the amount of hemolysis secured in each strength of solution. In making readings the following standard was adopted: Hemolysis was considered complete when no deposit of red cells followed precipitation with the centrifuge, the solution possessing a transparent red color. A solution showing a deposit of red cells and possessing a tinge of transparent red was considered as a minimal reaction of the red cells to the solution of sapotoxin to which they had been exposed. Inasmuch as whole blood was used, it was necessary to adopt a minimal red color as the point of minimal hemolysis, because of the straw color normally given by the presence of serum. Degrees of hemolysis between the minimal and maximal were read as good or fair depending upon the degree of hemolysis obtained. In cases where hemolysis was considered fair in one tube and laking failed to take place in the tube next higher in dilution, it was considered that the minimum point lay half way between the two dilutions. For instance, if tube 1:13,000 gave a reading of fair and the next higher dilution 1:14,000 failed to show any degree of laking the minimum point was considered to be a solution whose strength would be 1:13,500.

No difficulty was experienced because of clotting of the whole blood when placed in the testing tubes. The dilution of 20 c. mm. of blood as drawn from the wound in 1 c.c. of sapotoxin solution, and the short period of exposure, 5 minutes, to the hemolytic agent, evidently was enough to allay clotting. However, in certain cases of pronounced jaundice there was a tendency towards clotting, especially in the 1:13,000 solution used to determine complete hemolysis. The same blood was found to give constant results when a constant relationship was maintained between the quantity of blood, solution, time, and temperature.

#### EXPERIMENTAL RESULTS

The normal red blood cells in 99 cases was found to show an average maximal resistance of 1:13,769. The greatest resistance shown in the series was 1:13,500; the least 1:14,750. The range of variation was, therefore, 1,250 points. In checking new solutions 86 normal determinations were made upon H. W. The average maximum resistance of this series was 1:14,130; the greatest resistance 1:13,500; the least 1:14,500; the greatest variation 1,000 points. The average maximum resistance of the red blood cells for the entire number of normal readings, 185 cases, was 1:13,937. The average time for complete hemolysis to occur in a 1:13,000 sapotoxin solution was 10.7 minutes. The greatest length was found to be 18.5 minutes; the shortest 6.0 minutes (Table I). It is a difficult matter to determine the exact moment of complete laking or the moment of red cell destruction at any period without centrifuging the specimen, hence, the rather wide variations in the time concluded to repre-

sent the end reaction of hemolysis in the 1:13,000 solution. A 1:13,000 sapotoxin solution was chosen rather arbitrarily and early in the work because in most instances this strength solution failed to cause hemolysis in less than 5 to 7 minutes in normal samples of blood. This solution was therefore found convenient because it allowed time for making and completing the other readings. This time method, while inaccurate in itself, acted as a check upon the strength and activity of the other solutions.

For comparative purposes a series of experiments were made upon the resistance of washed red cells to sapotoxin solutions. This was deemed feasible inasmuch as the majority of present work upon blood is done upon washed cells suspended in some form of isotonic solution.

Samples of blood were drawn from the arm veins under aseptic precautions as in obtaining blood for the Wassermann reaction. From 15 to 20 c.c. of blood was usually obtained for the determinations and allowed to clot in the ice chest for 24 hours. It was found that a more compact clot formed and

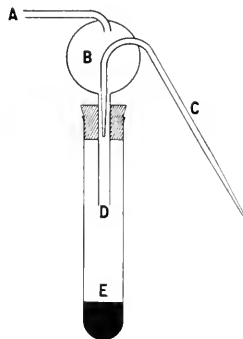


Fig. 2.—Air pump pipette used to withdraw serum from tubes containing coagulated blood. *A*, Connection with air pump. *B*, Large bowl connected with receiving tube *E* through tube *D*. *C*, Receiving tube. Reduction of air pressure in *B* and *E* permits fluid to flow through *C* into test tube *E*. This pipette, designed by Homer Wheldon, was found to be of special value in the handling of serums and small amounts of solutions.

relatively more serum extruded if the surface of the forming clot was freed from the edges of the tube. After 24 hours' standing the serum was removed from the clot by means of a specially prepared suction chamber pipette, Fig. 2. The clot was then broken up in saline solution and filtered through cotton; the cells greatly diluted with normal saline solution and centrifuged. Washings were continued until the supernatant fluid was free of any coloring matter. Usually 3 or 4 washings were sufficient to remove all serum and laked material. It was found that a 1:1 dilution of the free cells with 0.9 per cent saline solution gave practically a normal red cell count, hence such a dilution ruled out mass action that otherwise might have occurred.

In 12 samples of normal blood the average maximum resistance was found to occur in a 1:37,375 dilution of sapotoxin. In 21 samples of leucic blood the average resistance of the washed red cells was found to be 3,982 points lower

than normal, that is, laking of cells occurred in a solution of 1:41,357 strength. In pregnancy the average resistance of 5 cases was 1:37,100, and in 4 cases of jaundice 36,750. The resistance of 185 cases of normal whole blood (Tables I and II) was 13,936. In 34 samples of syphilitic blood the resistance was

TABLE I

## RESISTANCE OF NORMAL WHOLE BLOOD TO SAPOTOXIN SOLUTIONS

Showing the average resistance of the red cells in whole blood to sapotoxin solutions, based upon 99 determinations made on normal individuals and 86 controls made on H. W. Maximum and minimum resistances and maximum differences are shown. (Hem), strength of sapotoxin solution required to give a minimal hemolysis reading 5 minutes. (1:13), time required to bring about complete hemolysis in a sapotoxin dilution of 1:13,000. (Hb), hemoglobin percentage.

	NORMAL SUBJECTS. A.			CONTROLS ON H. W. B.			
	NO.	HEM	1:13	Hb	NO.	HEM	Hb
MAXIMUM RESISTANCE		13,500	18.5	95		13,500	95
MINIMUM RESISTANCE		14,750	6.0	85		14,500	90
MAXIMUM DIFFERENCE		1,250	12.5	10		1,000	5
AVERAGE	99	13,769	10.7	90.6	86	14,130	93

Average of A + B = 1:13,937 = Normal resistance.

TABLE II

## RESISTANCE OF WASHED AND UNWASHED RED CELLS TO SAPOTOXIN SOLUTIONS

Showing the degree of difference in resistance of red blood cells when freed from, and when in contact with, the normal fluids of the whole blood, in normal, jaundiced, syphilitic, tuberculous and pregnant individuals. (No), number of experiments. (Hem), strength of sapotoxin solution required to bring about a minimal laking of red cells within 5 minutes. (1:13), time required, in minutes, to bring about complete hemolysis in a sapotoxin dilution 1:13,000. (Hb), hemoglobin percentage.

	WASHED CORPUSCLES				UNWASHED CORPUSCLES				WASHED CELLS PLUS SERUM	
	NO.	HEM	COUNT	Hb	NO.	HEM	1:13	Hb	NO.	HEM
Syphilitic	21	41,357	5,086,190	81	34	14,699	11.7	85	4	16,437
Pregnancy	5	37,100	5,280,000	87	35	11,271	16.8	89	3	12,050
Jaundice	4	36,750	5,300,000	85	31	12,685	55.0	79.1	4	12,564
T. B. C.	3	35,000	5,346,000	87	24	12,375	13.1	81.6	3	13,025
Normal	12	37,375	5,100,000	86	185	13,937	10.7	90.6	10	14,050
Average	45	38,900	5,147,775	85	309	13,472	16.0	85.1	24	13,625
Difference = 25,428					+ 25,428					

\*Not on treatment.

14,669; in 35 cases of pregnancy 11,271, and in 31 cases of jaundice 12,685. Therefore, there is an average difference of 25,428 points in the resisting power of the red cells to sapotoxin solutions between the washed and the unwashed cells. Washing decreases the resisting power of the red cells in syphilis by 26,658 points; in pregnancy by 25,829; in jaundice by 24,065; in tuberculosis by 22,625, and in the normal by 23,438 points. Therefore, the serum normally about the red blood cells acts as a direct antihemolytic agent against sapotoxin. This protective action of the serum can also be shown by determining the resistance of washed cells which have been diluted with their own serum. As shown in Table II, the resistance of whole blood in 34 syphilitic

subjects was 1:14,699; in washed corpuscles 1:41,357. A 1:1 dilution of washed cells and serum in 4 cases gave on the average a resistance of 1:16,437, a resistance 1,738 points lower than whole blood and 24,914 points lower than washed cells. Figures are also given for similar determinations upon blood

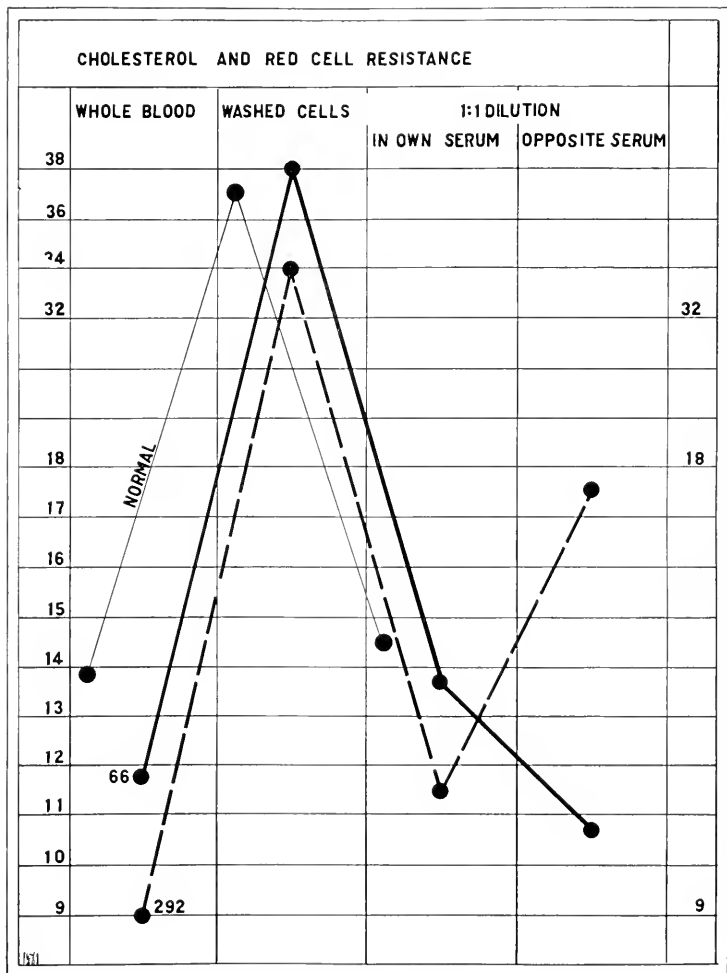


Fig. 3.—Curves showing relation of cholesterol and blood serum to red cell resistance. 66. Case No. 890, jaundiced; cholesterol 166. 292. Case No. 891, obstructive jaundice; cholesterol, 292. Normal. Curve for whole blood, washed cells and washed cells diluted 1:1 with their own serum. Note the effect of changing the serum relations about the cells of the two cases.



from jaundiced individuals. The relation of the red blood cells to whole blood, isotonic solutions and serum is shown graphically in Fig. 3.

Washed corpuscles are less resistant to the action of bichloride of mercury than unwashed cells. Butler<sup>2</sup> noted that the washing of red blood cells with a 0.9 per cent salt solution caused a distinct lowering of the resistance of the red cells, on the other hand, the washing of cells in a 4 per cent glucose solution did not cause a reduction in cell resistance. Such results lead to the conclusion that the decreased resistance resulting because of washing cells is due to the removal of the protecting material of the serum and to a disturbance of the osmotic conditions. Our results are in agreement with such statements.

Inasmuch as temperature affects chemical action, we chose 25° C. because of the convenience at which such a temperature could be maintained under varying conditions. Noguchi<sup>3</sup> noted that temperature changes greatly affected the reaction time of hemolytic agents; an increase in temperature decreases the laking time, while a reduction in temperature increases the time required to bring about the same degree of hemolysis. An experiment performed to demonstrate the relation of temperature changes upon the power of sapotoxin to bring about a minimal hemolysis gave the following results: Resistance readings at temperatures 15, 20, 25, 30, 35, 40 and 45 degrees centigrade were 1:10,000—1:13,250—1:14,100—1:18,000—1:21,000—1:24,000—and 1:26,000, respectively. Hence, an increased temperature decreases the strength of sapotoxin required to lake the same mass of red blood cells (20 c. mm.) in a given time. The maximal resistance as determined in a 1:13,000 sapotoxin solution for temperatures from 15° to 45° C. were 18, 14, 8, 5, 3, 2, 1.7, and 1 minutes, respectively. Hence, an increased temperature determines a more rapid rate of destruction of the red cells exposed to a given strength of solution.

#### SUMMARY AND CONCLUSIONS

I. A rapid method is described for the determination of the degree of resistance of the red blood cells to a specific hemolytic agent—sapotoxin. The average maximal resistance of the corpuscles in the whole blood of 99 individuals chosen as normals was a 1:13,769 strength sapotoxin solution. Eighty-six determinations on H. W. over the course of the experiments averaged a 1:14,130 strength solution. The average of all normal readings—185—was a 1:13,937 solution. The average length of time for complete hemolysis to occur in a 1:13,000 solution at a constant temperature—25° C.—was 10.7 minutes. The average hemoglobin as determined by the Tallquist hemoglobinometer was 91 per cent for all cases. Washed corpuscles from 12 normal cases suspended in isotonic salt solution were found to show a minimal degree of hemolysis in a 1:37,375 sapotoxin solution. Findings in leucic, pregnant, and jaundiced cases are also given. Washed corpuscles diluted 1:1 with normal saline solution gave practically a normal count, hence mass action was ruled out because of this dilution. Also, washed corpuscles diluted 1:1 with their own serum demonstrate practically the same resistance against sapotoxin as cells present in whole blood. The red blood cells normally show a remarkable degree of constancy in their resistance to a specific hemolytic agent.

II. Therefore, it may be concluded that the presence of the blood fluid about the red cells acts in such a manner as to resist the hemolytic action of sapotoxin.

## REFERENCES

- <sup>1</sup>Meltzer and Welch: Jour. Physiol., 1884, v, 225.  
<sup>2</sup>Butler: Quart. Jour. Med., 1913, vi, 145.  
<sup>3</sup>Noguchi: Jour. Exper. Med., 1906, viii, 337.

# LABORATORY METHODS

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## A TEST FOR EARLY RENAL INSUFFICIENCY\*

### PRELIMINARY PAPER

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FOR the past two decades many attempts have been made to study the renal function of patients who have demonstrable primary lesions of the kidneys, or lesions that affect the kidneys secondarily. As the result of all the methods known, many have claimed that the most generally valuable individual test is the estimate of a single sample of blood urea. Many investigators, however, have tried to find some test that would demonstrate an earlier renal involvement than that which can be shown by an already elevated blood urea. Thus the diagnostic determination of blood uric acid has been developed and it has been shown quite clearly that there may be an elevated blood uric acid in renal involvement long before the blood urea is above normal, because the kidneys excrete uric acid with the greatest difficulty of any known nonprotein substance. The determination of uric acid is of more value than urea in the early diagnosis of nephritis.

In cases in which the blood uric acid is marginal; that is, between 2.5 mg. and 3.5 mg. for each 100 c.c. the question often is raised of whether there is any significance in the results. It was thought possible to answer this question and at the same time demonstrate earlier lesions than could be shown by a single blood uric acid determination, if the patient were tested for his ability to excrete uric acid. Denis showed some years ago that patients with nephritis exhibit an accumulative effect of uric acid in the blood if fed on high purin diets, while normal persons have no change in the blood uric acid under these conditions. Upham and Higley pointed out the necessity of having patients on a constant purin intake in order to study and compare blood uric acid determinations. It seemed logical, therefore, if a patient were fed uric acid and there was a slight lesion of the kidneys too small even to give a very marked rise of blood uric acid with a normal diet, that it might be possible to demonstrate an early inability of the kidneys to excrete uric acid and thus determine a possible prenephritic condition. The test was carried out as follows:

The patient was put on a purin-free diet (milk) for three days and the amount of uric acid excreted in the urine was determined for the third twenty-four hours. The method of Folin and Wu was used throughout this work. Three-fourths of the weight of the patient was ascertained and calculated for the weight of body fluids. Enough uric acid was given by mouth to cause a rise in the blood uric acid of about 2.5 mg. for each 100 c.c. if the patient failed

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to excrete any of it. This usually meant the administration of about 2 gm. of pure uric acid. A determination was then made of the uric acid in the blood. The uric acid excreted during the next twenty-four hours was determined; at the end of this time a second blood uric acid determination was made and compared with that made at the time the drug was administered. It is evident, therefore, that there is a check on any uric acid that might be lost by changes in the rate of absorption in the gastrointestinal tract.

Three patients were tested in the manner described. None of the three presented blood or urinary findings by the usual methods sufficient to warrant the diagnosis of renal involvement, yet all three had a decided hypertension with practically no other physical findings. In one case the patient excreted, prior to the administration of uric acid, 0.4 gm. in twenty-four hours. At the time he was given uric acid, the blood contained 2.5 mg. for each 100 c.c. At the end of the next twenty-four hours the patient had excreted 0.34 gm. of uric acid, and the blood determination showed a concentration of 5.8 mg. for each 100 c.c. Since the other two cases ran parallel, only one will be cited. The patient excreted 0.5 gm. of uric acid in twenty-four hours, prior to the administration of the drug and showed a blood uric acid of 2.8 mg. for each 100 c.c. At the end of twenty-four hours the blood uric acid concentration had not changed and about 70 per cent of the uric acid was recovered in the urine. The first case was interpreted as a prenephritic condition. The other two must, for the present, be considered as essential hypertensions without renal involvement.

The uric acid used for these tests was prepared from human urine by the usual method. Several other cases were tested as here outlined; a commercial product of uric acid was used. There was neither rise in the uric acid of the blood nor of the urine, indicating that the failure might be due to the fact that the uric acid was probably not absorbed in the gastrointestinal tract, at least not as uric acid. This result is in keeping with observations made by investigators in the past.

Caffeine citrate was used in other cases, and here I was able to obtain calculated rises in the blood or urine according to whether the individual was normal or nephritic. From this it would seem that caffeine is excreted as uric acid or piles up as uric acid in the blood in cases of nephritis or else some other substance is formed that gives a blue color by the Folin and Wu method. The dose used was about 1 gm. of caffeine citrate in capsules at three one-hour intervals just prior to the ingestion of a meal.

The method by which caffeine is excreted is still a question of controversy. Sollman asserts that it is completely and readily absorbed, that very little is excreted as such, and that from 10 to 40 per cent of the substance loses its methyl groups and is excreted as dimethylxanthin or monomethylxanthin; up to 80 per cent is excreted as urea, practically none as uric acid. Cushney asserts that the uric acid of the urine is not increased by the ingestion of caffeine. On the other hand Greene believes that caffeine is demethylated in the body and forms xanthin which is excreted in the manner in which any animal xanthin is excreted, namely as uric acid. Schittenhelm found that feeding caffeine increases the amount of uric acid in the urine. Taylor con-

TABLE I  
NO LESIONS OF THE KIDNEY

CASE	DIAGNOSIS OF NEPHRITIS (CONNER)	BLOOD URIC ACID 1	BLOOD URIC ACID 2	DIFFERENCE BETWEEN 1 AND 2	URINE URIC ACID 1	URINE URIC ACID 2	DIFFERENCE BETWEEN 1 AND 2	REMARKS
325661	Syphilis	2.4	2.4	±0.0	0.47	0.69	+0.22	Treatment for syphilis 0.4 gm. caffeine*
343023	Neurosis, migrating, syphilis	1.3	2.1	-1.8	0.41	0.40	-0.01	
342157	Pulmonary tuberculosis	3.2	2.4	-0.8	0.25	0.86	+0.61	
342901	Bronchial asthma, prolapse of ovaries	2.1	1.9	-0.2	0.31	0.37	+0.06	
341789	Artificial vagina	2.0	2.8	+0.8	0.15	0.27	+0.12	Treatment for syphilis
340455	Epilepsy, central nervous system syphilis	2.5	2.9	+0.4	0.29	0.38	+0.09	
342960	Leukorrhea	1.8	2.0	+0.2	0.38	0.62	+0.24	
342904	Metrorrhagia, neurosis	2.2	1.9	-0.3	0.19	0.81	+0.62	
341076	Chronic sinusitis	2.4	1.7	-0.7	0.68	0.89	+0.21	Treatment for syphilis
316534	Central nervous system syphilis, cystitis	2.3	2.3	±0.0	0.76	0.62	-0.14	
340982	Barbican, pneumonia	2.0	2.2	+0.2	0.40	0.42	+0.02	

\* Full dose 0.8 gm. caffeine.

TABLE II  
 LESIONS OF THE KIDNEY (?)

CASE	RENAL DIAGNOSIS	CLINICAL DIAGNOSIS (OTHER THAN RENAL)	BLOOD URIC		BLOOD URIC		DIFFERENCE BETWEEN 1 AND 2		URINE URIC		DIFFERENCE BETWEEN 1 AND 2		REMARKS
			ACID 1	TRIC 1	ACID 2	TRIC 2	ACID 1	TRIC 1	ACID 2	TRIC 2	ACID 1	TRIC 1	
34104	Hypertrophy of prostate	Probably slight nephritis	3.1	3.1	2.3	0.0	-0.8	0.0	0.77	0.17	+0.17		Twelve-hour specimen urine 0.4 gm. caffeine* Eighteen-hour specimen urine
34373	Exophthalmic goiter	Probably mild chronic nephritis with arteriosclerosis	3.8	3.8	3.2	0.6	-0.6	0.73	0.51	-0.22			
342739	Pericarditis	Possibly mild psychonephritis	1.7	1.7	1.9	0.2	+0.2	0.30	0.25	-0.05			
342897	Bronchial asthma	Probably mild nephritis	3.0	3.0	2.8	0.2	-0.2	0.58	0.58	+0.00			
34069	Myocarditis	Possibly nephritis	3.0	3.0	3.0	0.0	+0.0	0.56	0.54	-0.02			Twelve-hour specimen urine 0.4 gm. caffeine* Eighteen-hour specimen urine
34158	Chronic constipation; psychoneurosis	Possibly mild nephritis	1.4	1.4	2.5	1.1	+1.1	0.65	0.74	+0.11			
224717	Dental infection, nervousness	No lesion of the kidney ?	1.4	1.4	1.7	0.3	+0.3	0.28	0.50	+0.22			
342665	Anemia, gastritis	Probably mild nephritis	1.4	1.4	1.8	0.4	+0.4	0.28	0.52	+0.04			
340597	Ikterus	No lesion of the kidney ?	2.4	2.4	3.1	0.7	+0.7	0.19	0.46	+0.27			Twelve-hour specimen urine 0.4 gm. caffeine* Eighteen-hour specimen urine
338792	Hypertension	Probably some chronic nephritis	2.6	2.6	3.3	0.7	+0.7	0.55	0.46	-0.09			
433090	Achylia gastrica, pyorrhea	Possibly mild chronic nephritis	3.6	3.6	3.8	0.2	+0.2	0.67	0.42	-0.25			

\* Full dose 0.8 gm. caffeine.

TABLE III  
DEFINITE LESION OF THE KIDNEY (CLINICALLY)

CASE	GENERAL DIAGNOSIS (CONSER)	BLOOD URIC ACID 1	BLOOD URIC ACID 2	DIFFERENCE BETWEEN 1 AND 2	URINE URIC ACID 1	URINE URIC ACID 2	DIFFERENCE BETWEEN 1 AND 2	REMARKS
338961	Chronic nephritis, arteriosclerosis	6.0	2.8	3.2	0.12	0.20	-0.12	0.1 gm. caffeine, eighteen hour specimen of urine
342561	Left pyelonephritis with chronic nephritis	3.8	3.3	-0.5	0.70	0.99	0.29	
341707	Hydronephrosis with stones, nephritis	1.9	2.5	+0.6	0.26	0.39	0.13	
340546	Infected hydronephrosis	2.1	3.1	+0.7	0.13	0.42	0.19	
341689	Mild nephritis, arteriosclerosis, hypertension	3.0	3.3	+0.3	0.83	1.00	0.17	
339900	Mild nephritis	2.2	3.0	+0.8	0.26	0.28	0.02	
341621	Mild chronic nephritis, hypertension, arteriosclerosis	2.1	2.8	+0.7	0.90	0.81	-0.09	
342111	Chronic nephritis, hypertension	3.1	3.8	+0.7	0.12	0.10	-0.02	
342499	Nephritis, arteriosclerosis, hypertension	1.6	2.7	+1.1	0.77	0.54	0.23	0.1 gm. caffeine
342063	Chronic nephritis	2.7	3.7	+1.0	0.39	0.53	0.14	Gout?
338749	Mild nephritis	3.2	2.8	-0.4	0.11	0.18	0.07	0.1 gm. caffeine
341339	Chronic nephritis, hypertension	2.8	2.8	0.0	0.57	0.65	0.08	
340860	Mild nephritis	2.1	2.3	+0.2	0.57	0.98	0.41	
341846	Chronic nephritis	2.5	2.8	+0.3	0.70	0.12	-0.58	Eighteen hour specimen urine
342234	Right kidney infected	2.0	2.4	+0.4	0.52	0.68	0.16	Eighteen hour specimen urine
343473	Mild nephritis	2.2	3.0	+0.7	0.62	0.36	-0.26	

\*Full dose 0.8 gm. caffeine.

cluded that coffee added to the diet increases the output of uric acid quite markedly, and explained it on the basis of the caffeine in the coffee. Benedict repeated, to some extent, the work of Taylor and concluded that caffeine at least when ingested as coffee increased the output of uric acid in the urine.

This caffeine test has been used in a number of cases which were studied clinically and diagnosed in relation to the condition of the kidney. The cases were divided into three groups, those without lesions of the kidney, those with questionable lesions from a clinical study, and those with definite lesions clinically. The results are shown in Tables I, II, and III. It may be seen that in general the caffeine test checked very nicely the clinical findings. Three patients (Table I) are marked exceptions to this statement. All were on intensive antisiphilitic treatment and it is possible that they had very slight kidney lesions, which must be quite common with intensive treatment with mercury and arsenic, but cannot be detected clinically. One other exception to the clinical findings is seen in Table III. A man with a left pyelonephritis had a free output of uric acid, which may be explained on the basis of a competent right kidney. A case diagnosed mild nephritis with some question of gout showed practically no increase in the output of uric acid, but rather a little diminution in the second blood specimen taken. This finding is interesting in view of the fact that uric acid is believed by some observers to be retained in the tissues in cases of gout.

Recently McLean and Wesselow have used a urea concentration test showing that earlier renal involvements may be demonstrated than by a simple blood urea estimation. The test consists, briefly, in feeding the patient 15 gm. of urea and determining the amount excreted in the urine in two hours' time. It remains to be seen whether this test furnishes more information than a single uric acid determination, and certainly from a theoretical standpoint it could not show as early a renal involvement as the proposed uric acid concentration test outlined in this paper. These tests were performed many months before the publication of the work of McLean and Wesselow, in an attempt to demonstrate the rôle of renal insufficiency in essential or idiopathic hypertension. The pressure of other investigations, however, has made it impossible to carry on the work at the present time and this note is published in the hope that others interested in such cases will give the test a trial and will perhaps obtain a series that may be compared with other renal functional tests.

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## EDITORIALS

### *Syphilology and Clinical Synthesis*

OSLER said, once upon a time, that Jonathan Hutchinson was the greatest generalized specialist or specialized generalist in medicine because he knew syphilis in all of its ramifications. He also said, by way of corollary, that if a man knows syphilis he knows medicine.

Syphilis is one of the few diseases in which interest never fails and about which a special literature grows at a rather even rate. The curve of interest in the Great Pox does not seem to have a form resembling that of the old Baltimore and Ohio Railroad—or the Erie; it is not a malarious curve. Other infectious diseases have their ups and downs. Syphilis follows a straight line. The general and uniform interest in the disease is reflected in the fact that the Wassermann reaction has become a matter of routine in clinical studies.

But while the laboratory test is an aid to the physician, its introduction and widespread use has perhaps overemphasized the mere laboratory side of diagnosis to the extent that many men trust rather to it than to their observational skill in studying cases of syphilis or of cases in which syphilis is a possible factor. There is a tendency to forget that the Wassermann reaction like any other laboratory test is a thing which adds something to a clinical

history, and that it is the entire clinical history upon which a diagnosis rests. Not only diagnosis but prognosis is prone to suffer from the general clinical disregard which follows too complete dependence on the laboratory. So as Stokes and Busmann say, reversal of the Wassermann reaction while desirable should not be the primary aim of the therapy. Symptomatic response with arrest of the process, and the giving of as much treatment as tolerance permits are the chief considerations.

Stokes has evidently been through the so-called laboratory stage for he has emerged from that "monastic phase in response to a new appreciation of the enduring worth and ultimacy of clinical research." The laboratory has become his assistant, rather than his master. The critical problem of the next half century of syphilology, he says, is not so much the extension of laboratory knowledge now so highly developed, as the application of the knowledge already existent, to the clinical problems of the disease. No reasonable man would seek to minimize the worth of the laboratory contribution. But a decade of transformations has been superimposed on the clinical syphilology of Fournier, Hutchinson and Morrow as two fluids of different densities may be superimposed the one upon the other in a test tube. What is needed now is a mixture, a solution. The day of the *pousse café* is past.

Stokes' experience has taught him that syphilis is no longer a problem of the dermatosyphilographer alone, and that the day of the syphilographer who can combine in his sole personality both the technical attainments and the wealth of general and detail knowledge that make him master of the disease, is past. He verifies Osler.

He has made a list of the examinations on which, at one time or another, his department relies for a diagnosis or information on the status of a syphilitic infection, and a glance at this list will show how illusory is the hope that syphilis will ever yield to a single diagnostic key. The rapid modification of clinical knowledge under the pressure of new technical diagnostic and therapeutic methods has induced a peculiar state of disorganization in the syphilologic realm. True syphilology is, for the time being, lost in the mazes of an analytic era and the syphilologic workshop is crowded with personalities each so intent on the part which he contributes that he can give no thought to the whole. The syphilologist of the future is as yet in a phase of mental dissociation suggesting the unfortunate condition of the Miss Beauchamp of Morton Prince's study. Synthetic studies are needed.

In the present state of affairs, with the trend of medicine more and more to specialism, groups have grown up and the group system enlarges itself just because synthetic studies are so obviously needed. But as yet the system is very imperfect except in a few instances. When the system is perfected every doubtful case, whether of syphilis or what-not, will be the subject not only of sporadic clinical notes from each division of the group, but of a round table conference at which with the clinicians the laboratory man will have a seat. Then the previous analyses can be studied and synthetically estimated. In such conferences each specialist can get and will get a bird's-eye view of a subject matter and of methods that are largely foreign to him, and a clinical perspective will gradually develop. There will be less flatness

in office and laboratory work and clinical stereosecopy will be developed. The laboratory will no longer be a monastery from which edicts are issued, but will become a cooperative part of the group from which will come opinions and suggestions which will be discussed and evaluated by all the members.

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—P. G. W.

### *The Spread of Bacterial Infection*

TOPLEY<sup>1</sup> has made an interesting experimental study of an epidemic among mice. A certain number of these animals were fed cultures of *B. Gaertner* and from time to time healthy mice were added to the infected community. Effort was made to be sure that the additions were in health. They were taken from batches of from six to eighteen which had been kept in cages for two or more weeks and in which there was no evidence, either by disease or death, of the presence of an infection. These healthy mice were added to the infected cage from time to time. The experiment continued through three hundred eighty-eight days, during which time seven hundred eighty-two mice were added and seven hundred twenty-eight deaths occurred. It was found that the percentage of deaths increased when the fresh animals were added during the ascent of a mortality wave. When fresh animals were added while the mortality was on the increase not only was death more frequent among the additions, but also increased among those already in the cage and which had survived other epidemic waves. The author says: "In the present study it has been shown that if normal mice are added from day to day to an infected population they will all eventually succumb, provided that further normal mice are added at a certain rate. The period of survival of any given batch of mice will vary according to the time at which they are introduced to the cage. If their entrance coincides with the early part of the rise of an epidemic wave, as judged by a mortality curve, their survival will be short. If they are introduced during the latter part of the decline of such a wave they will live, on the average, much longer, longer indeed than mice subsequently added to the cage."

By frequent addition of susceptible material to an infected community, an epidemic may apparently be prolonged indefinitely. If this be applicable to epidemics among human beings, it is quite evident that quarantining an infected community is desirable and beneficial not only for protecting those outside such community, but with the object of limiting the deaths within the community itself. We are of the opinion that we observed something like this in our cantonments during the late war. It was certainly evident that with each additional accession of fresh material to an infected camp the number of cases and the number of deaths increased and the increase involved not only those who came into the camp, but those who were originally in it.

<sup>1</sup>*Jour. Hygiene*, 1921, xix, 350.

Topley states his conclusions as follows: "(1) If susceptible mice be continuously added to an infected population the spread of infection will continue over a long period of time. There is no evidence that this period has a limit. (2) When susceptible mice are added continuously and at a constant rate to an infected population, the spread of infection, as judged by a mortality curve, is propagated in regular recurring waves. These waves are most easily observed by noting the fluctuations in the total cage-population. It seems probable that the period of these fluctuations will be found to depend on the rate of addition of susceptible individuals, but this point has still to be determined. (3) The actual deaths may occur in large groups, with intervals during which deaths are few and far between, or they may fall in a succession of smaller groups, increasing and diminishing in size to form the larger waves. In all cases there is this tendency for the occurrence of such small groups of deaths with definite maximal points. There would seem to be two fluctuating processes, the one superimposed upon the other. (4) The average survival-time of mice added to the cage, and their chance of ultimate survival if no more susceptible mice are introduced, vary according to the phase at which they are added. If they gain entrance to the cage during the rise of a wave they are unlikely to live for long. If they are introduced during the fall of a wave their chances of survival are greatly increased, and they will usually outlive mice which are added at a later date but at a time before the commencement of the next wave. (5) The rate of extinction of a population, among which infection is actively spreading, will be far less rapid if they are kept isolated, than if further susceptible individuals continuously gain access to them. A proportion of the infected population, which would have survived indefinitely under the former circumstances, will die under the latter. (6) The ultimate survivors among such a population have not escaped infection, but have successfully resisted it. A considerable proportion of them are harboring the causative parasite in their tissues."

We are tempted to theorize after reading the above mentioned report, but have concluded that theory had better be omitted until more experimental work along this line has been done. Incidentally, however, we cannot help recalling the fact that we would not have known of yellow fever in Cuba had it not been for the susceptible accessions that were constantly coming into the Island.

—V. C. V.

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NO. 9

## ORIGINAL ARTICLES

### RELATION OF DIFFERENTIATION AND LYMPHOCYTIC INFILTRATION TO POSTOPERATIVE LONGEVITY IN GASTRIC CARCINOMA\*

BY WM. CARPENTER MACCARTY, M.D., AND ARTHUR E. MAHLE, M.D.,  
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IN 1912 one of us (MacCarty)\*\* published an article reporting the relation of regional lymphatic glandular involvement to postoperative longevity in gastric carcinoma. The series of specimens which was studied consisted of 200 resected portions of the stomach. The location and relative size of the gastric lymphatic glands were indicated on diagrams and each gland was examined microscopically for carcinoma. The specimens were divided into three groups,



Fig. 1.—No glandular involvement.



Fig. 2.—Involvement of some glands.



Fig. 3.—Involvement of all glands.

i. e., Group I, specimens with no glandular involvement; Group II, specimens with some glandular involvement; and Group III, specimens with all of the glands involved (Figs. 1, 2, 3).

The investigation of that series revealed the following facts:

1. Carcinomatous ulcers in the stomach varied from 1 centimeter to 14 centimeters in diameter and from 1 centimeter to 5 centimeters in depth.

\*Read before the American Association for Cancer Research, New York, April, 1920.

\*\*MacCarty, Wm. Carpenter, and Blackford, J. M.: Involvement of Regional Lymphatic Glands in Carcinoma of the Stomach, *Ann. Surg.*, June, 1912.

2. The average ages in relation to glandular involvement at operation were: Group I, 50.8 years; Group II, 51.7 years; Group III, 46 years.
3. The duration of "dyspepsia" in the three groups, respectively, was 8.5 years, 8+ years, and 8+ years.
4. The average duration of symptoms was 17.2 months, 12.5 months and 17.4 months.
5. The average loss of weight was 25 pounds, 28.4 pounds and 42.5 pounds.
6. The hospital mortality was 7.6 per cent, 11.6 per cent, and 18.7 per cent.
7. The size of regional lymphatic glands in gastric carcinoma bore no apparent relation to the size of the primary lesion in the stomach.
8. The size of a regional lymphatic gland was no criterion of the presence or absence of carcinoma.
9. Gross diagnosis of the presence or absence of carcinoma in lymphatic glands was of no value excepting in advanced carcinoma of the glands.
10. The duration of clinical symptoms bore no apparent relation to the size, and extent of involvement in lymphatic glands.
11. The average age and sex, at operation, bore no direct relation to the glandular involvement.
12. The average loss of weight increased with the increase in amount of glandular involvement.
13. The immediate hospital postoperative mortality was in direct proportion to the amount of glandular involvement.
14. The subsequent, or posthospital, mortality was in direct proportion to the amount of glandular involvement.

	GROUP I	GROUP II	GROUP III	
15. Average age of patients	51.3 yrs.	49.7 yrs.	45.8 yrs.	
Youngest	30 "	29 "	30 "	
Oldest	72 "	68 "	66 "	
16. Average length of life of the whole series in different decades	29-40 2.7 yrs.	40-50 2.5 yrs.	50-60 2.3 yrs.	60-72 3 + yrs.

At the expiration of eight years after the first investigation, we were able to determine all of the facts relative to the longevity in association with perfect specimens for study in 99 cases\* out of the original 200 cases.

The following facts were recorded upon the 99 cases of the present investigation:

1. Cases with no glandular involvement have a much greater average length of postoperative life.
2. No cases with glandular involvement lived over 8 years.
3. Six per cent of cases without glandular involvement lived over 10 years. Two per cent without glandular involvement lived 11 years or over. One per cent without glandular involvement lived 13 years after operation.
4. No case between 29 and 60 years of age lived over 10 years. Nine per cent of cases between 60 and 72 years of age lived over 10 years. Four and

\*Many of the specimens of the original series of 200 had been so mutilated during other studies that they were not in the best condition for this investigation.

seven tenths per cent of cases between 60 and 72 years of age lived over 12 years.

5. The average age of cases with complete glandular involvement is 5 years younger than those cases without glandular involvement.

6. The average age of the youngest cases is the same regardless of glandular involvement.

7. The eldest case with glandular involvement is 5 years younger than the eldest without glandular involvement.

8. The average length of postoperative life is greater between 29 and 40 years of age and 60 and 72 years of age.

In spite of the fact that the length of postoperative life was in inverse relation to the degree of glandular involvement, there was a sufficient number of exceptions to demand further investigation. One case lived over five

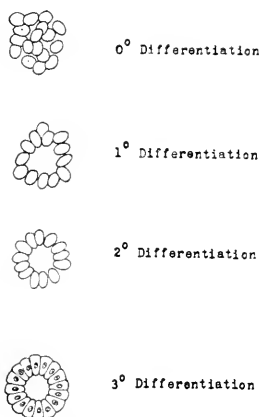


Fig. 4.—Degrees of cellular differentiation based on the stages of differentiation of specific tissues during embryologic development. In the stage without differentiation the cells are merely arranged in masses. In the first degree of differentiation the cells assume the general alignment of the adult tissue but without arrangement of the axes of the cells as in the adult tissue cells. In the second degree of differentiation the axes are parallel or form radii of a sphere or circles. In the third degree the cells assume the adult morphology of the units of the specific tissue.

years and one lived over nine years in spite of the fact that all of the lymphatic glands were involved. The same was true of cases with partial glandular involvement. The question arising from these facts is: What are the factors controlling longevity in these exceptional cases? Doubtless there are many contributing factors to longevity but there are at least two which can be studied with a fair degree of accuracy which may be sufficient to advance our knowledge of the body's defensive mechanism against the destructive character and activities of neoplasms. In this report, cellular differentiation, and lymphocytic infiltration are considered. There is a general unwritten law in biology which indicates that the power of cellular reproduction is in inverse relation to the degree of cellular differentiation unless the differentiation be for the specific purpose of reproduction. By the term differentiation in biology we

mean structural change for specific function. For convenience of study the degrees of differentiation have been classified according to the visible changes which can easily be recognized in the normal evolution of specific tissues. The differentiation has been divided into three degrees (Fig. 4). The cells of carcinoma sometimes attempt differentiation and it has been thought that this attempt might be seen in the cases which lived unusually long after gastric resection. The specimens were, therefore, studied from this standpoint.

The next factor, as a possible part of the defensive mechanism, is lymphocytic infiltration. The degrees of lymphocytic infiltration are arbitrarily fixed as follows: The first degree, no immediate contact of the lymphocytes with the cancer cells, the lymphocytes being discovered only after careful search of the microscopic fields; second degree, occasional contact of the lymphocytes with the cancer cells; third degree, extensive contact of the lymphocytes with the cancer cells. While this is an arbitrary division of degrees of lymphocytic reaction, the extremes only may be considered to be of possible value since the personal equation, as to the second degree, would be variable in the hands of different observers.

The findings, in so far as the degree of differentiation and lymphocytic infiltration are concerned, were charted with the age, sex, and years of post-operative life.

The three groups were studied separately (see charts) with the following results:

PERCENTAGES ALIVE IN GROUPS (99 CASES)

				GROUP I	GROUP II	GROUP III
Percentage which lived over	1 year			78.7	50	50
" " " "	2 years			60	21.4	30
" " " "	4 "			33	8.9	20
" " " "	6 "			21	5.3	10
" " " "	8 "			12	3.5	10
" " " "	10 "			6	0	0
" " " "	11 "			2	0	0
" " " "	13 "			1	0	0

It may be seen that 78.7 per cent of gastric carcinomata without glandular involvement lived over 1 year; 60 per cent of gastric carcinomata without glandular involvement lived over 2 years; 33 per cent of gastric carcinomata without glandular involvement lived over 4 years; 50 per cent of gastric carcinomata with glandular involvement lived over 1 year; 21-30 per cent of gastric carcinomata with glandular involvement lived over two years; from

				29-40	40-50	50-60	60-72
Percentage alive over	1 year	in decades		46	67	67	76
" " " "	2 years	" "	" "	38	32	32	42
" " " "	4 "	" "	" "	23	17	17.5	19
" " " "	6 "	" "	" "	15	14	8	9
" " " "	8 "	" "	" "	7	3.5	8	9
" " " "	10 "	" "	" "	9	0	0	9
" " " "	12 "	" "	" "	0	0	0	4.7
" " " "	13 "	" "	" "	0	0	0	0



8.9 to 20 per cent of gastric carcinomata with glandular involvement lived over 4 years, or 80.91 per cent died before the end of the fourth year after resection; from 3.5 to 10 per cent of gastric carcinomata with glandular involvement lived over 8 years; and 6 per cent of gastric carcinomata without glandular involvement lived over 10 years.

From these figures it may be seen that the younger the host the shorter the life expectancy after resection for gastric carcinoma regardless of glandular involvement.

## CELLULAR DIFFERENTIATION

Average length of postoperative life in the different groups with no differentiation	I 3.5 years	II 1.4 years	III 2.8 years
Average length of postoperative life in the different groups with differentiation	4 "	1.68 "	2.6 "
Percentage of cases with no differentiation	57.5 "	25 "	20 "
Percentage of cases with differentiation	42.5 "	75 "	80 "
Decade .....	29-40	40-50	50-60
Percentage with no differentiation.....	53.8	25	35
Percentage with differentiation.....	46.2	75	65
Average length of postoperative life in all cases with no differentiation.....	2.8 years		
Average length of postoperative life in all cases with some differentiation.....	2.55 "		

GROUP I

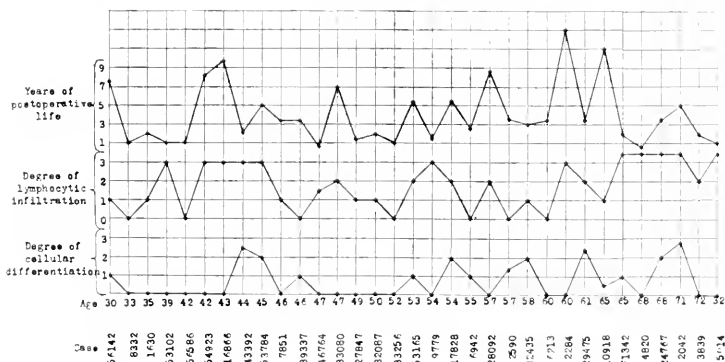


Fig. 5. Group I.

The facts from these figures may be briefly summarized as follows:

1. Individuals with no glandular involvement and showing differentiation lived 14 per cent longer than those with no glandular involvement and no differentiation.

2. Individuals with partial glandular involvement with differentiation lived 20 per cent longer than those without differentiation.

3. In individuals with complete glandular involvement differentiation was not associated with increased longevity.

4. Differentiation was more frequent in association with glandular involvement (Group II, 42.6 per cent; Group III, 70 per cent).

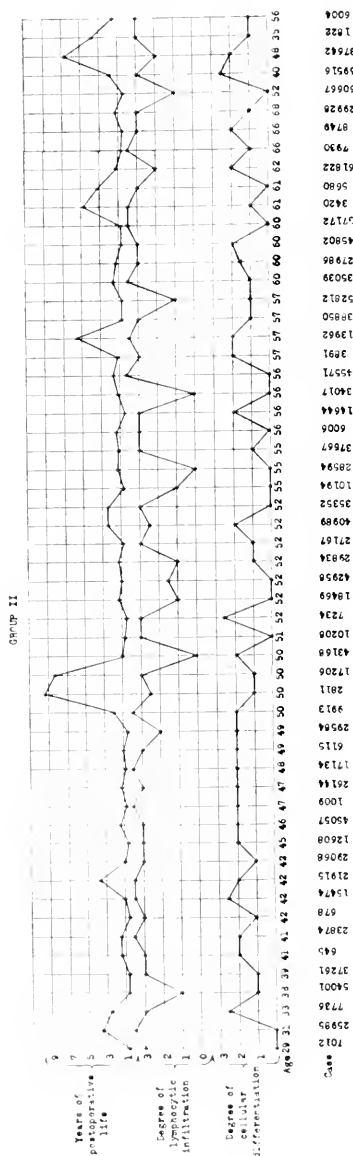


Fig. 6.—Group II.

5. Differentiation was most frequent between the ages of 40 to 50 years and least frequent between 29 to 40 years.

6. In the whole series of cases the average length of life was less (8.9 per cent) with differentiation than without differentiation.

LYMPHOCYTIC INFILTRATION

	I	II	III
Average length of postoperative life in cases with 2 and 2+ lymphocytic infiltration...	5.3 years	4.1 years	4.5 years
Average length of postoperative life in cases with 3 and 3+ lymphocytic infiltration...	4.37 "	1.7 "	2.5 "
Average length of postoperative life in cases no lymphocytic infiltration.....	4.2 "	1.3 "	0.0 "
Average length of postoperative life in all cases with some lymphocytic infiltration			2.7 "
Average length of postoperative life in all cases with no lymphocytic infiltration			2.1 "
Percentage of cases with no lymphocytic infiltration		21	5
" " " " 2 or 2+ "		16	8.9
" " " " 3 or 3+ "		36.3	73.2
" " " " 1 or 1+ "		24.2	12.5
Decade .....	29-40	40-50	50-60
Percentage of cases with on lymphocytic infiltration	7	3.5	17.5
" " " " 1 "	23	14	20
" " " " 2 "	7	10	11
" " " " 3 "	61	71	47

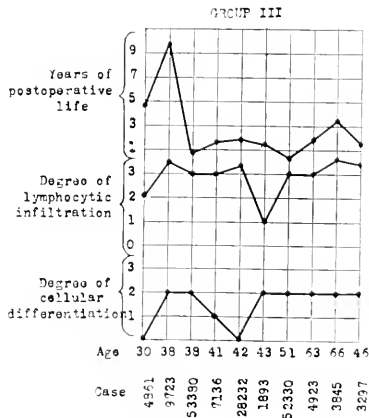


Fig. 7—Group III.

A study of these figures reveals the following facts in this series:

1. Cases of gastric carcinoma without glandular involvement plus extensive lymphocytic infiltration have the greatest average length of postoperative life (23 per cent longer than when associated with glandular involvement).

2. Regardless of glandular involvement the presence of lymphocytic infiltration is associated with a 23 per cent longer postoperative life.

3. Extensive lymphocytic infiltration is more frequent in association with glandular involvement than it is without glandular involvement.

4. Regardless of glandular involvement extensive lymphocytic infiltration is most frequent between 40 to 50 years and 60 to 72 years.

#### COMBINATION OF LYMPHOCYTIC INFILTRATION AND DIFFERENTIATION

Average length of postoperative life with	I	II	III
2 and 2 differentiation and 3 lymphocytic infiltration	4 years	1.5 years	3 years
Average length of postoperative life with			
no differentiation and lymphocytic infiltration	1.6 "	1.5 "	0 "

From a study of these figures the following conclusions from the series may be made:

The cases of gastric carcinoma with the combination of lymphocytic infiltration, differentiation and no glandular involvement lived 150 per cent longer than the cases with no differentiation and no lymphocytic infiltration.

#### SUMMARY

1. The average length of postoperative life is 7.5 per cent greater in cases with differentiation than in those without differentiation.

2. There is a much greater percentage of cases in Group I without differentiation.

3. There is a much greater percentage of cases in Group III with differentiation.

4. The greatest frequency of cases with no differentiation occurs in cases between 29 and 40 years of age.

5. The greatest frequency of cases with the greatest amount of differentiation occurs in cases between 40 and 50 years of age.

6. The average length of postoperative life is greatest in cases without glandular involvement plus lymphocytic infiltration.

7. The cases without glandular involvement but with lymphocytic infiltration live 124 per cent longer than those without lymphocytic infiltration.

8. Cases with glandular involvement lived 146 per cent longer when there was lymphocytic infiltration than did those with no infiltration.

9. The highest percentage of cases, without lymphocytic infiltration, were those with no glandular involvement.

10. The highest percentage of cases, with lymphocytic infiltration, were those with glandular involvement.

11. The highest percentage of cases with marked lymphocytic infiltration occurred between 60 and 72 years of age and 40 and 50 years of age.

12. The highest percentage of cases with no lymphocytic infiltration occurred between 50 and 60 years of age.

13. The average length of postoperative life in cases with differentiation and lymphocytic infiltration combined is 82 per cent greater than that without differentiation and lymphocytic infiltration combined.

## THE ALKALI RESERVE OF THE BLOOD PLASMA DURING PROTEIN SHOCK\*

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THE shock produced by the intravenous injection of foreign proteins recently advocated in the nonspecific treatment of chronic infections processes is frequently associated with symptoms suggestive of an acidosis. In order to determine a decrease in the blood alkalies of animals shocked in this manner a series of investigations have been made of which this communication constitutes a report.

Observations upon the alkali reserve of the blood, during surgical shock, have been made by L. J. Henderson,<sup>1</sup> Yandel Henderson,<sup>2</sup> Crile,<sup>3</sup> Caldwell and Cleveland,<sup>4</sup> Guthrie,<sup>5</sup> McElroy,<sup>6</sup> Cannon,<sup>7</sup> and others. As surgical and toxic shock are in all essential features, identical, it is well to review briefly the observations of these investigators. Henderson<sup>2</sup> and coworkers have frequently reported acidosis in surgical shock of animals, produced by experimental means, and his acapnia theory of acidosis based upon these experiments is quite well known. Crile<sup>3</sup> has observed acidosis present in various clinical conditions including shock. Guthrie<sup>5</sup> found a decrease in the reserve alkalinity of the blood in certain cases of experimental shock, but much of the decrease observed occurred after pronounced symptoms of shock had become manifested and that good recovery of cerebral function was possible in shock without a change in the hydrogen-ion or the plasma alkalinity which strongly indicated to the author that the cerebral manifestations of shock are not due to changes in the blood reaction. McElroy<sup>6</sup> found in experimental shock, a gradual decrease in the alkali reserve of the blood. His experiments led him to the conclusion that acidosis was not a causative factor in the experimental shock studied, and he states that in primary experimental acidosis, where there is a marked lowering of the alkali reserve to a degree lower than that observed in shock, no marked change in the condition of the animal was produced, also that the injection of sodium bicarbonate into animals in shock, was without beneficial action, although the reserve alkali was restored. McElroy found that shock may be induced even while the alkaline reserve is maintained by the injection of sodium bicarbonate. Cannon's<sup>7</sup> studies included forty-seven cases of low blood pressure in soldiers during the recent war. In some cases the low blood pressure was due to shock alone, while in others it was complicated by hemorrhage or gas-bacillus infection. The relationship of acidosis, blood pressure, respiratory changes and the blood sugar received particular attention in these cases. Cannon also observed the effect of anesthesia and operation upon an existent acidosis and lower blood pressure and the influence of alkaline treatment in cases of ex-

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treme acidosis. In forty per cent of Cannon's cases the mean blood pressure was below 60 millimeters of mercury, and all showed a condition of acidosis. A close parallelism in the drop of the carbon dioxide capacity of the blood, with a drop in the mean blood pressure was found. In ten cases of uncomplicated shock the average carbon dioxide capacity was 41 volume per cent. Upon the basis of some experiments carried out upon soldiers in shock, Cannon highly recommends the preliminary administration of alkalis in order to combat the extreme acidosis that follows operations on men in surgical shock.

#### TECHNIC

In these experiments, dogs were used chiefly. These dogs were kept in the laboratory kennel upon a well balanced diet, for a period not less than a week. The carbon dioxide capacity of the plasma was determined according to the technic of Van Slyke.<sup>8</sup> The animals were bled from the jugular vein, by means of a syringe and the blood immediately mixed with neutral potassium oxalate, and covered with liquid paraffin. The blood was tested previous to the production of the shock and at regular intervals following. Secondary proteoses and typhoid bacteria were the toxic substances used to produce shock and were given intravenously.

#### EXPERIMENTS

TABLE I  
Dog 1, weight 5 kg.

EXPERIMENT TIME	INJECTION	C.C. OF CO <sub>2</sub> CHEMICALLY BOUND BY 100 C.C. PLASMA
9:00 A.M.	1.0 gm. proteoses	50.4
9:10 A.M.		
2:00 P.M.		25.8
3:00 P.M.		30.0
6:00 P.M.		38.1
9:00 A.M.		50.0

Table I shows a marked acidosis in a dog, following proteose shock. Similar experiments were repeated upon a series of eight dogs with slight variations in results. Therefore it is considered sufficient to present in tabular form the average figures obtained from the eight experiments.

TABLE II  
Average weight of eight dogs, 5.1 kg.  
Average dose of proteoses, 0.2 gms. per Kg.

TIME INTERVALS	INJECTION	C.C. OF CO <sub>2</sub> CHEMICALLY BOUND BY 100 C.C. PLASMA
Before injection	Proteoses	54.8
10 minutes later		
2 hours later		32.4
8 hours later		40.4
24 hours later		45.0
48 hours later		53.2

Both Tables I and II show a marked decrease in the carbon dioxide capacity of the plasma of dogs following shock produced by the intravenous injection of toxic proteoses. Seven of the nine dogs used in these two ex-

periments, showed a drop in the carbon dioxide capacity of the plasma. In one dog included in Table II, receiving the same amount of proteoses proportionately as the others, there was practically no change in the alkali reserve of the blood. However, this animal presented definite evidence of acute shock. In spite of this unexplained variation in the one animal, the table shows a rapid and continuous decline in the alkaline reserve of the blood. The animals usually recover from this condition of acidosis in less than twenty-four hours. The symptoms presented by the animals were vomiting, diarrhea, restlessness, dilated pupils, prostration, low blood pressure. One of the animals died during the night following the shock. When a decrease in the alkali reserve was below 20 per cent the animals usually showed severe clinical symptoms and the animal which died showed a carbon dioxide capacity decrease of 56.7 to 18.3. Blood pressure tracings were made on several dogs, and in these animals there was a drop in the pressure following the injection of the proteoses which was closely associated with a drop in the alkali reserve of the plasma. This is similar to the findings of Cannon in surgical shock. The failure of one of the animals in the series to show a decrease in the alkali reserve following the proteoses injection remains unexplained. However, Guthrie<sup>3</sup> reports an irregular production of acidosis in dogs during surgical shock.

The results obtained upon proteose shock in dogs suggested the use of other toxic proteins upon the alkali reserve of the blood, and in the following experiments, typhoid vaccine was employed intravenously to produce a severe shock.

Below are tabulated the average figures of seven animals shocked by the intravenous injection of typhoid bacteria.

TABLE III  
Average weight of seven dogs, 6.5 Kg.  
Average dose per Kg., 40,000,000 dead typhoid bacteria.

TIME INTERVALS	INJECTION	C.C. OF CO <sub>2</sub> CHEMICALLY BOUND BY 100 C.C. PLASMA
Before injection	Typhoid bacteria	55.3
10 minutes later		29.3
2 hours later		40.2
8 hours later		53.8
24 hours later		48.6
48 hours later		

There is a marked drop in the carbon dioxide capacity of the plasma of dogs receiving an intravenous injection of typhoid bacteria as shown in Table III. In one dog of this series, the carbon dioxide capacity fell from 60.5 volume per cent to 19.5 volume per cent. This animal died the night following the injection of the bacteria. Another one of the dogs, in which the carbon dioxide capacity had dropped to 26.02 volume per cent, died. There was marked prostration in all of these animals. The symptoms were more severe following the typhoid than the proteose injections. The typhoid-shocked animals showed more marked diarrhea, the stools containing a large amount of blood. There was a greater diminution of the alkali reserve of the plasma in the animals receiving the typhoid vaccine, than those shocked by proteoses.

## HUMAN CASES

Having demonstrated changes in the alkali reserve of the plasma of dogs following protein shock, it was considered advisable to study the reaction of the plasma following the therapeutic administration of toxic proteins in patients.

A negro woman, aged forty, suffering from chronic arthritis, with carbon dioxide capacity 55.8 volume per cent before an intravenous injection of twenty million dead typhoid bacilli. The injection was followed by chill, headache and general malaise, and a rise in temperature of two degrees. One hour after the injection, the plasma carbon dioxide capacity was 45.9. Similar experiments upon a group of eight patients were repeated with similar results. All of the patients showed a slight decrease in the carbon dioxide capacity of the plasma following this form of shock.

## THE EFFECT OF THE ADMINISTRATION OF SODIUM BICARBONATE

From the observations of the above experiments it was thought desirable to determine the effect of the administration of sodium bicarbonate upon these reactions when given intravenously to the animals before and after the production of a shock.

TABLE IV

Average weight of four dogs, 6.2 Kg.  
Average dose of proteoses, 0.2 gms. per Kg.

TIME INTERVALS	INJECTIONS	C.C. OF CO <sub>2</sub> CHEMICALLY BOUND BY 100 C.C. PLASMA
Before injection	2 gms. sodium bicarbonate	60.3
10 minutes later		
30 minutes later		75.8
45 minutes later	Proteoses	
1½ hours later		40.2
8 hours later		50.4
24 hours later		62.6

TABLE V

Average weight of four dogs, 7 Kg.  
Average number of typhoid bacilli per Kg., 100,000,000.

TIME INTERVALS	INJECTION	C.C. OF CO <sub>2</sub> CHEMICALLY BOUND BY 100 C.C. PLASMA
Before injection	2 gms. sodium bicarbonate typhoid bacteria	69.1
15 minutes later		
35 minutes later		76.4
1 hour later		
1½ hours later		35.6
10 hours later		59.7
22 hours later		63.4

Eight dogs were taken, and the carbon dioxide capacity of the blood determined. The dogs were given sodium bicarbonate and the rise in the alkali reserve of the blood noted. In four of these dogs, proteoses were given and in the other four, typhoid vaccine. The blood plasma carbon dioxide capacity of these dogs showed a drop to a point far below the capacity before the ad-



ministration of the alkali. Clinically, the eight animals showed a distinct protein shock, with little or no change in the clinical symptoms, but the carbon dioxide capacity did not reach as low a level as in the untreated. In the tables are shown the composite results of these experiments.

The next experiments were made to determine the effect of the administration of alkali after the production of shock in dogs. It will suffice to give the following representative experiment which shows the results obtained in a group of five dogs.

TABLE VI  
Weight of dog, 6 Kg.  
Number of typhoid bacteria, 400,000 per Kg.

TIME INTERVALS	INJECTION	C.C. OF CO <sub>2</sub> CHEMICALLY BOUND BY 100 C.C. PLASMA
Before injection		56.1
18 minutes later	Typhoid vaccine	
40 minutes later		50.3
1 hour later	2 gms. sodium bicarbonate	
1 hr. 20 min. later		40.5
9 hours later		45.6
20 hours later		52.4

In Table VI is represented a very rapid decrease in the alkali reserve of the plasma of a dog after vaccine shock with a subsequent rise after the administration of sodium bicarbonate. All the animals showed severe clinical symptoms after the injections of the typhoid vaccine. Several of the dogs showed an apparent improvement of certain clinical symptoms immediately following the alkali treatment. Dyspnea was less, blood pressure higher, vomiting and diarrhea not so severe, as in the animals not receiving alkali. However, all the toxic symptoms were not relieved, and several of the dogs remained prostrated for several hours.

#### DISCUSSION

From these experiments it is evident that in protein shock, there is a decrease in the alkali reserve of the blood plasma. These findings correspond with the observations of Crile, Cannon, Wright and others, upon the alkali reserve of the blood following surgical shock and in infectious diseases. From these experiments, the decrease in the alkali reserve in infectious disease, is probably due to the liberation of toxic proteins into the circulation. The sources of the toxic proteins, being a bacteremia or the incomplete autolysis of the necrotic cells of tissues and inflammatory exudates. These experiments indicate that proper attention should be paid to the alkali reserve of the blood in the treatment of toxemic conditions and infectious diseases, particularly typhoid fever and pneumonia, as in these conditions large amounts of toxic proteins gain entrance into the circulation.

As a result of the improvement of certain distressing symptoms in dogs, after the administrations of alkalis, it would seem advisable to administer alkali in the treatment of infectious diseases and other toxemias for the relief of respiratory and circulatory symptoms so frequently found in these conditions.

## CONCLUSION

1. The alkali reserve of the blood plasma is greatly decreased in shock, following the intravenous injection of toxic proteoses and typhoid vaccines in dogs and in human cases.

2. There was found a definite relationship between the decrease in the alkaline reserve of the plasma and the lowered blood pressure in toxic shock.

3. When the alkali reserve of the blood falls below thirty volume per cent, following protein shock, the animal's life is in danger.

4. The administration of sodium bicarbonate preliminary to the injection of a toxic protein retards the fall of the blood alkalies to this critical point.

5. When the alkali reserve has been lowered in protein shock, it may be restored by the intravenous administration of sodium bicarbonate, which apparently relieves distressing symptoms.

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## STUDIES ON THE RESISTANCE OF THE RED BLOOD CELLS \*

### II. THE RESISTANCE OF THE RED BLOOD CELLS IN DISEASE TO THE HEMOLYTIC ACTION OF SAPOTOXIN

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IN a previous communication a rapid method was given for the determination of the resistance of the red blood cells to sapotoxin solutions.<sup>1</sup> In brief the method consisted of exposing a given amount of whole blood, 20 c. mm., for a period of 5 minutes to various strengths of sapotoxin solution at a constant temperature—25° C. With this method the red cells of normal whole blood of 185 determinations showed an average minimal degree of hemolysis in a 1:13,937 sapotoxin solution. It was also shown that the resistance of washed cells was markedly less than that of those suspended in normal blood fluids. Such results led to the conclusion that the blood fluids acted in such a manner as to protect or raise the resistance of the red blood cells to the hemolytic action of sapotoxin.

The present paper deals with the relation of red cell resistance in various of the common diseases to the hemolytic action of sapotoxin. Brief mention is made concerning the relation of cholesterol to the hemolytic action of sapotoxin inasmuch as the cholesterol content of the blood in certain of the diseases was found to vary in proportion to variations in red blood cell resistance.

#### EXPERIMENTAL RESULTS

##### I. PREGNANCY AND THE RESISTANCE OF THE RED BLOOD CELLS

While studying the resistance of the red blood cells of patients suffering from pulmonary tuberculosis we were impressed by the great degree of resistance offered by two women both of whom were pregnant. No. 77 (8 months pregnant) demonstrated a resistance of 1:10,500, the other, No. 78 (6 months pregnant) 1:10,000. The average resistance of 34 tuberculous patients was 1:12,375, an increase above the normal of 1,562 points, hence, tuberculosis and pregnancy each tend to accentuate the resistance of the red blood corpuscles to sapotoxin solutions.

The laking reaction of red cells in whole blood to sapotoxin solutions was determined upon 41 cases of pregnancy. In all 139 determinations were made during the latter days of pregnancy, labor, and the 4th, 8th, and 12th days postpartum. Thirty-five tests made within two weeks of delivery gave an average resistance of 1:11,271 solution. The time for complete hemolysis to occur in a 1:13,000 solution was 16.8 minutes. The average hemoglobin was 89 per cent as determined by the Tallquist hemoglobinometer. (See Table I and Fig. 1.)

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Ten cases were studied during the period of labor. The average strength of sapotoxin solution required to hemolyze the red cells in five minutes was a 1:10,050 solution. Complete laking occurred in the 1:13,000 solution in 19.2 minutes; average hemoglobin 90 per cent. Hence, during parturition there is an increased resistance to sapotoxin solutions of 1,221 points above that of the last two weeks of the prepartum period and 3,887 points increase over the

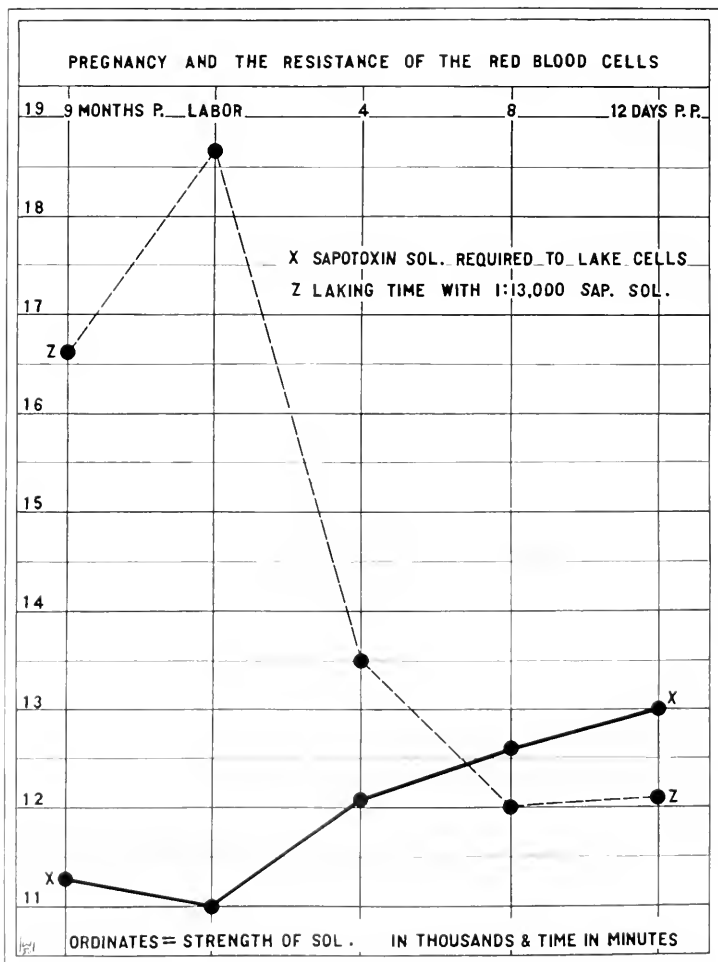


Fig. 1. Curves indicating the resistance of the red cells during pregnancy and the first 12 days postpartum.

average normal. The length of time required for complete laking of the red cells in a 1:13,000 solution was also increased by 2.4 minutes.

Thirty-three determinations made on the fourth day postpartum gave an average resistance strength of 1:12,060 solution. The average laking time in a 1:13,000 solution was 14.1 minutes; hemoglobin averaged 88 per cent. Hence the blood cells were less resistant by 1,010 points than during the period of delivery; 789 points less than the prepartum period and 1,877 points higher than the average normal. The time for complete laking in the 1:13,000 solution was decreased by 5 minutes over the time required at the time of delivery and 2.6 minutes less than the prepartum determinations.

TABLE I  
PREGNANCY AND RED CELL RESISTANCE TO SAPOTOXIN SOLUTIONS

	NO. CASES	RESISTANCE	1:13,000	Hb.
Prepartum	35	1:11,271	16.8	89
Late r	10	1:11,059	19.2	90
Postpartum				
4th Day	33	1:12,060	14.2	88
8th Day	31	1:12,581	12.2	90
12th Day	30	1:13,000	11.8	87
Average of all	139	1:11,953	14.9	89
Normal	185	1:13,957	10.7	91

Resistance, Strength solution showing minimal hemolysis. 1:13,000.  
Time in minutes for complete hemolysis to occur. Hb., Hemoglobin.

On the eighth day postpartum 31 tests gave an average resistance strength of 1:12,581 solution; a decrease of 1,310 points below the prepartum period and 1,356 points above the average normal. The average time for complete laking in the 1:13,000 solution was 12.2 minutes, or 4.6 minutes less than the prepartum period. Average hemoglobin, 90 per cent.

Thirty determinations made on the twelfth day postpartum gave an average laking strength of 1:13,000 solution, a decrease of 1,729 points below the prepartum period and 937 points above the average normal. The average laking time for a 1:13,000 solution was 11.8 minutes; a decrease of 5 minutes below the prepartum period.

Early pregnancy appears to increase the resistance of the red blood cells to sapotoxin solution. The average resistance of 18 cases of pregnancy between the third and seventh month was a 1:12,139 solution; an increase of 1,798 above the average normal, or 868 points lower than the average resistance for the period just prior to parturition. The average hemoglobin was 86.4 per cent and the average complete hemolysis in a 1:13,000 solution was 13.1 minutes.

Inasmuch as obstetrical cases usually leave the hospital 14 days after delivery it was not possible to carry the observations over a longer period of time. At the end of twelve days the resistance of the red blood cells is materially reduced below that of the prepartum period; however, it still shows a slight increased resistance above that of the average normal.

The work of Hymanson and Kahn<sup>2</sup> shows that the mean total lipid content of the maternal blood is 0.475, cholesterol 0.219 per cent. The blood from the newborn infant gives practically identical readings. According to

Bloor and Knudson<sup>2</sup> there is a constant relation between the free cholesterol and cholesterol esters of human blood. In whole blood the average percentage of cholesterol in combination as esters is 33.5 per cent, and in the plasma 58 per cent of the total cholesterol content. In pregnancy the values for cholesterol esters are high. Hypercholesterolemia is common in pregnancy although not invariable. The relation of gallstones to pregnancy is of common observation.

## II. MALARIA AND THE RESISTANCE OF THE RED BLOOD CELLS

Inasmuch as malaria is accompanied by more or less severe destruction of the red blood cells by the malarial plasmodium, it was considered of interest to test the resistance of the erythrocytes before and after the administration of quinine. The relation of red cell destruction and quinine in the production of "black-water fever" of necessity brings up the question of an active hemolysis in such cases.

Ninety-eight blood resistance determinations were made upon 38 malarial patients. Twenty-three positive malarial cases upon whom tests were made prior to the administration of quinine gave an average minimal hemolysis reaction in a 1:14,380 sapotoxin solution. Apparently, therefore, the presence of the infection does not greatly alter the resistance of the erythrocytes as there is but 443 points difference in such cases and the average normal.

The first day following the taking of massive doses of quinine (15 to 60 grs.), 35 blood determinations gave an average minimal resistance of 1:15,022. On the second or third day following quinine medication the resistance of the erythrocytes fell to an average of 1:15,417, or a loss of 1,037 points below the average prior to the taking of the quinine and 1,470 points below the average normal. Following the initial reduction, the resistance of the red cells gradually returned and demonstrated a minimal hemolysis in a solution of 1:14,750 at the end of a week's medication.

Following the withdrawal of quinine, the resistance of the red cells gave an average reading of 1:14,409, a resistance equivalent to that demonstrated during the time of malarial invasion and prior to the taking of quinine. "Black-water fever" did not develop in any of our cases, hence, no information was obtained relative to that point.

As a check upon the above experiments, the effects of quinine on seven nonmalarial persons was determined. These persons received the same dosage of quinine and were tested the same as the malarial patients. The average resistance of these controls prior to the taking of quinine was 1:14,142. Within 24 hours after taking quinine the resistance of the red cells was increased to a 1:13,893 solution; a figure within the range of normal determinations. At no time during the entire week of medication did the average resistance move out of the range of normal variations. The withdrawal of quinine had no apparent effect upon the red cells as the average resistance at such a time was 1:13,937 or the same as the average normal. (See Table II and Fig. 2.)

Hemoglobin is apparently but little affected by quinine, although our series of patients did demonstrate an average gain of 7.3 per cent in hemo-

globin following heroic quinine medication. Such a gain may be accounted for because of the cessation of red cell destruction by the malarial plasmodium.

Lovelace,<sup>1</sup> in a study of 514 cases of hemoglobinuric fever found that malarial infection stands in a direct causal relation to "black-water fever".

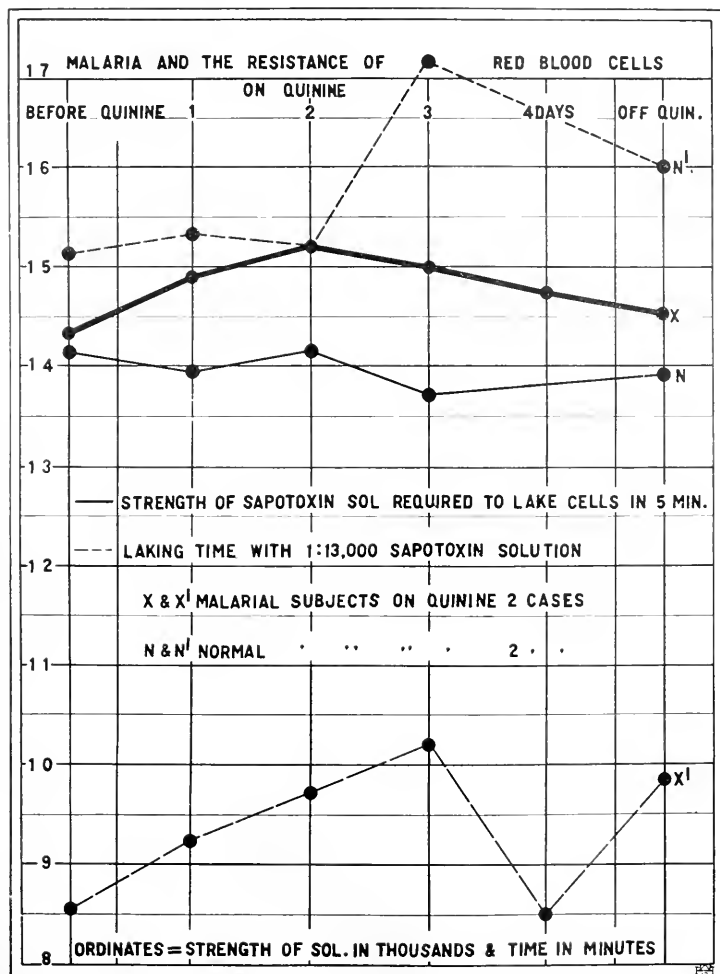


Fig. 2.—Curves showing the effect of quinine on the resistance of normal and malarial blood cells to sapotoxin. The 2 nonmalarial cases received the same treatment as the 2 malarial cases. The legends of the figure explain the curves.

TABLE II  
MALARIA AND RED CELL RESISTANCE TO SAPOTONIN SOLUTIONS

	NO. CASES	RESISTANCE	1:13,000	Hb.
Malaria	23	1:14,380	11.6	86
1st Day on Quinine	35	1:15,022	8.5	89
During Week on Quinine	32	1:15,242	8.9	89
Off Quinine	8	1:14,409	9.3	90
Average of all	98	1:14,763	9.5	89
Normals	185	1:13,937	19.7	91
NON-MALARIAL PATIENTS ON QUININE				
Before Quinine	7	1:14,142	15.0	86
1st Day on Quinine	7	1:13,893	15.3	88
During week on Quinine	5	1:13,944	5.7	88
Off Quinine	4	1:13,937	16.00	86
Average of all	23	1:13,979	13.0	87

The administration of quinine either in large or small doses, was, in his cases, an invariable antecedent of the hemoglobinuric condition. Brem,<sup>5</sup> on the other hand, does not believe that quinine acts as an etiologic factor, either predisposing or exciting. However, Brem does believe that previous attacks of malaria, especially of the estivo-autumnal type, do act as predisposing causes of hemoglobinuric fever. He also advanced the theory that the malarial organism probably excited the production of an acute hemolysin, which in turn destroyed the red cells. The views of Cardamatis<sup>6</sup> concerning the etiology of black-water fever agree with those of Lovelace, namely, quinine and malaria. Cardamatis found the mortality in cases of black-water fever treated with quinine 23.6 per cent, while in cases treated without quinine the rate of mortality was but 7.5 per cent.

The relation of paroxysmal hemoglobinuria to syphilis is of interest in that syphilis like malaria is the result of a protozoan parasite. Hemoglobinuria is also known to occur in various protozoan invasions of animals. In such cases the development of an autolysin or hemolysin gives rise to the destruction of the subject's own corpuscles. As will be shown more fully later, the degree of resistance of the red cells to laking agents is materially altered in cases of syphilis not under treatment. This loss of resistance, or increased fragility, is especially marked when the washed cells are studied.

Butler<sup>7</sup> reports that the resistance of the red corpuscles to salt solutions is normal in malarial cases. Sahli<sup>8</sup> in discussing this point says: "The determination of the power of resistance of the red blood cell to hypotonic injury is not sufficient to settle all questions in reference to their resisting power, since Chvostek, 1899, in contrast to Murin, found that in paroxysmal hemoglobinuria the power of resistance of the red blood cells to salt solutions is normal, while they show but slight resistance to mechanical injury (shaking) and to stasis within the body."

### III. TUBERCULOSIS AND THE RESISTANCE OF THE RED BLOOD CELLS

Reports relative to the resistance of the red cells in tuberculosis to salt solutions are conflicting. It is more than probable that factors other than



tuberculosis are to be considered in such cases: the degree of anemia, cachexia, malignancy, jaundice, syphilis and pregnancy all affect the resistance of the red cells. Hence, a disturbance of tissues other than those affected by tuberculosis may account for the variations in results that have been reported. Pregnancy, as previously shown, does increase the resistance of the red blood corpuscles to a striking degree. This observation has also been reported by other observers.

The average resistance of erythrocytes from 24 patients suffering from pulmonary tuberculosis was 1:12,375, an increase of 1,562 points above the average normal. The time for complete hemolysis to occur in a 1:13,000 solution was 13.1 minutes or an increase of 2.4 minutes above the average normal. The hemoglobin determinations averaged 81.6 per cent (Table III).

Two patients (Nos. 77 and 78) suffering from pulmonary tuberculosis were pregnant, one 5 months, the other 8 months. No. 77 (8 months) demonstrated a resistance of 1:10,500; an increase of 3,437 points above the normal and 1,875 points above the average for the 24 tuberculous cases. No. 78 (5 months) gave a resistance of 1:10,000, an increase above the average normal of 3,937 points or an increase above the average for the tuberculous patients of 2,575 points. In the light of the results obtained from the study of pregnancy these two cases show not only an increased resistance of the red blood cells to sapotoxin because of the tuberculous condition but also because of pregnancy.

Cases Nos. 523 and 827 were both tuberculous and luetic and were receiving treatment accordingly. The resistance of the red cells in these two cases was 1:14,000 and 1:13,750, respectively. Hence Case No. 523 demonstrated a reading which fell within the range of the average normal, but demonstrated a decrease of 1,625 points below the average resistance of the 24 tubercular cases. Case No. 827 showed a resistance of 1:13,750; an increase of 187 points above the normal average of 1:13,937, or a decrease of 1,375 points below the average for tuberculous patients. The low hemoglobin may in part account for these findings. Hence, lues in some way reduces the resistance of the red blood cells in cases suffering from this disease in conjunction with tuberculosis. (See Section V.)

TABLE III  
PULMONARY TUBERCULOSIS AND RED CELL RESISTANCE TO SAPOTOXIN

	NO. CASES	RESISTANCE	1:13,000	HB.
All cases	24	1:12,375	13.1	82
Lues and pregnancy	19	1:12,312	11.4	83
Tuberculosis and lues	2	1:13,875	15.8	68
Tuberculosis and pregnancy	2	1:10,250	23.3	83
Normals	185	1:13,937	10.7	91

#### IV. TYPHOID FEVER AND RED BLOOD CELL RESISTANCE

Conflicting results have been reported relative to the degree of resistance of the red blood corpuscles in cases of typhoid fever. The usual results seem to be about the same as those for normal individuals. However, it appears that

TABLE IV  
TYPHOID FEVER AND RED CELL RESISTANCE TO SAPOTOXIN

	CASES	RESISTANCE	1:13,000	Hb.
All Stages of Typhoid fever	19	1:13,513	10.8	85
Normals	185	1:13,937	10.7	91

during the period of convalescence there is a slight increase in the resistance of the red cells, also an increase in the cholesterol content of the blood.

The resistance of the red blood cells to sapotoxin solutions was determined in 19 positive cases of typhoid. The average resistance of the series was 1:13,513, or an increase of 424 points—readings within the normal range of variation. The average time for complete hemolysis to occur in a 1:13,000 solution was 10.8 minutes. The average hemoglobin was 85 per cent. The greatest resistance shown was in Cases 127 and 297 in which the resistance was 1:12,000 in each. Both patients were running high temperatures but otherwise quite comfortable. The greatest loss of resistance occurred in Case 763 who was doing badly and soon died. His resistance was 1:15,750.

Hemolysis determinations at times showed an increased resistance of the cells during the period of convalescence; however, variations at any time were not great. Therefore, typhoid fever appears to alter but little the resistance of the red blood cells to the laking action of sapotoxin solutions. Conditions complicating typhoid fever seem to alter the resistance of the red cells, but no definite conclusions could be derived from our study. (Table IV.)

#### V. SYPHILIS AND THE RESISTANCE OF THE RED BLOOD CELLS

The resistance of the red blood cells to sapotoxin solutions was determined in 61 cases of syphilis. The average resistance in 34 positive cases prior to medication was 1:14,699, or 762 points lower than the average normal. Fifty-six determinations made upon 23 patients under mercurial treatment alone gave an average red cell resistance of 1:13,659. This represents an increase of 1,040 points over the average untreated patients and 278 points increased resistance over the average normal. The average resistance of 24 determinations upon positive cases of syphilis under a treatment of mercury and iodides was 1:14,750. The addition of iodides to the treatment, therefore

TABLE V  
SYPHILIS AND RED CELL RESISTANCE TO SAPOTOXIN SOLUTIONS

	CASES	RESISTANCE	1:13,000	Hb.
Positive lues				
no treatment	34	1:14,699	10.2	82
On mercurials	56	1:13,659	14.0	88
On mercury and iodides	13	1:14,750	10.7	84
On iodides	11	1:13,977	9.4	86
Average	114	1:14,271	11.7	86
CONTROLS ON POTASSIUM IODIDE		15 M. SAT. SOL. THREE TIMES A DAY		
Normal	4	1:14,625	11.2	90
On iodides	4	1:15,375	14.6	90
Normal	185	1:13,937	10.7	91

caused a decrease of 1,091 points in the resistance of the blood cells below the average during mercurial treatment alone, or a reduction of 813 points below the normal average. (Table V and Fig. 3.)

The effect of potassium iodide on the red cell resistance was determined on 4 normal non-luetic persons. These four cases received 15 mm. three times a day for

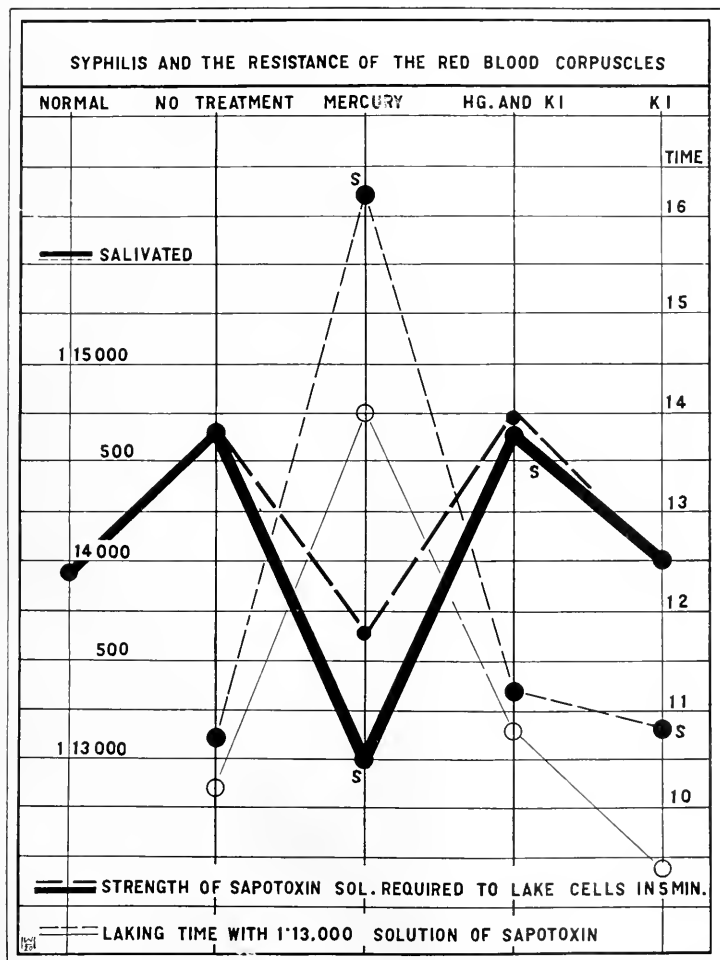


Fig. 3.—Curves showing the effect of mercury and the iodides on the resistance of the red cells of syphilitics. The results of salivation in two cases are shown by curves so indicated. The remaining curves represent the average reactions of all cases studied under the various treatments.

two days. The determinations were made from two to three hours after the last dose. The average normal resistance of these men was 1:14,625—698 points below the average normal. Determinations made after the medication gave a decrease of 750 points in the resistance determinations. The average hemoglobin determination was 90 per cent. Hence the experimental use of iodides resulted the same as in luetic cases following the administration of mercurials.

#### VI. LEAD POISONING

Seven cases of lead poisoning gave an average resistance of 1:14,321. Five of these cases were receiving belladonna and magnesium sulphate treatment; these gave an average resistance of 1:14,321 solution. The 2 cases untreated averaged a resistance of 1:15,125, or 812 points greater than the normal average.

#### VII. JAUNDICE AND THE RESISTANCE OF THE RED BLOOD CELLS

Chauffard<sup>9</sup> working with Ribierre's method for the determination of the resistance of the red cells to hypotonic salt solutions, found that obstructive jaundice could be differentiated from other types, designated by him as "hemolytic", by the fact that in obstructive jaundice the resistance of the red cells was raised, while in hemolytic jaundice it was lowered. He also reports that the red cells show a lessened resistance in pernicious anemia with jaundice, and in the so-called "Hereditary jaundice of Minkowski". According to Sommer<sup>10</sup> the red cell resistance is definitely lowered in diseases accompanying elevated blood pressure, and especially in hemolytic icterus.

Hypercholesteremia is invariably present in cases of cholelithiasis. Gorham and Myers<sup>12</sup> found the cholesterol content to vary in such cases. Cases of congenital jaundice show a marked lowering of the cholesterol content of the blood. De Langen<sup>11</sup> reports that gallstone complaints are seldom seen among the Javanese and that the cholesterol content of the blood is only one half the quantity found in European blood. The bile also has a low cholesterol content.

Unquestionably the liver and spleen in mammals in some way aid in the destruction of red cells. However, the removal of the spleen does not prevent the formation of bile pigments. The bile pigments are derived from hemoglobin; hence, it appears that alterations in the functioning of the liver give rise to alterations in the resistance of the erythrocytes to hemolytic agents. Certainly one of the functions of the liver is to keep the various constituents of the altered red cells innocuous for blood cell stroma and hematoporphyrins are, in themselves, toxic and hemolytic in nature. If the bile ducts are ligated, bile pigment and other salts rapidly accumulate in the blood of mammals and birds, on the other hand, ligation of the portal vein and hepatic artery does not result in an accumulation, and bile pigments do not occur in the urine. Any means which leads to the setting free of hemoglobin in the blood causes an increase in the excretion of bile pigments. Injection of certain chemicals and inhalations of arseniureted hydrogen cause marked hemolysis, which may be carried to the point of hemoglobinuria and an in-

crease in the secretion of bile. Injections of hemoglobin produce the same results. Hence, red cell destruction means increased bile pigment formation, and if for any reason the flow of bile from the ducts is hindered, the liver is no longer able to rid itself of its own action and jaundice results.

Jaundice, however, appears to result because of two widely different causes; (a) by occlusion of the bile passages, and (b) by specific hemolytic processes. Perhaps the underlying causes are the same in both cases. Hemolytic jaundice may be the result of accumulation of materials in the blood in excess of the power of the liver to properly care for them in the manufacturing of bile. The cholesterol metabolism is at fault, and this allows the unchecked action of hemolytic agents upon the red blood cells. The cholesterol of the bile may be in part derived from the food and in part from the destroyed blood cells. However, the bile is able to dissolve more cholesterol than is usually found in it. This power of solution of bile depends on the presence of bile salts and specifically upon the cholic acid radicle of the salts. The cholic acid portion of such salts is closely associated with cholesterol, and because of this the cholesterol is maintained in solution. Bile salts are in themselves hemolytic and act similar to the saponins which form easily dissociable compounds with cholesterol. Therefore, it is probable that cholesterol unites in a similar way with the hemolytic group of the bile salts thereby forming a soluble compound in the bile. If such is true, the accumulation of bile salts and a reduction of cholesterol may be, in part, at least, the cause of certain types of jaundice.

The average resistance of the red blood cells as determined by 31 tests upon 27 jaundiced patients was a 1:12,685 sapotoxin solution. This gives an average increased resistance of 1,252 points above the average normal (Table VI). The average hemoglobin was 79 per cent. It was found practically impossible to determine the time for complete hemolysis in a 1:13,000 solution. Usually the blood coagulated and hemolysis was suspended; this was especially true for blood drawn from patients with gallstones. The average for 19 such determinations was 55 minutes. This, however, cannot be considered as at all accurate.

Fifteen positive cases of gallstones gave an average resistance of 1:10,033, or an increase above the average normal of 3,904 points.

Five cases of hemolytic jaundice gave an average resistance of 1:18,600 or a decreased resistance of 4,663 points below the average normal and 8,567 points below the average for obstructive jaundice cases.

Eleven miscellaneous cases of jaundice not definitely diagnosed gave an average resistance of 1:13,182. The variations in resistance were great in this series of cases.

Four cases of cirrhosis of the liver gave an average resistance of 1:14,500, —563 points below the normal average.

The red cells from 4 patients suffering from obstructive jaundice when washed gave an average resistance of 1:36,750 sapotoxin solution. The average resistance of 12 determinations made upon blood from normal individuals was 1:37,375. Hence, there is but little difference in the resistance of washed

cells whether taken from normal or jaundiced cases. However, red cells exposed to their own blood fluids show a great degree of resistance in cases of obstructive jaundice (1:10,033). The relation of the blood fluids to the resistance of the red cells is shown graphically in Table VII. From the table it will be noted that the washed red cells of Case X when placed in the serum of Case Y offered a greater resistance than when placed in their own serum. On the other hand, the washed cells of Case Y when suspended in the serum of Case X which contained less cholesterol offered a less resistance to sapotoxin than when placed in their own serum. Hence, the contents of the blood fluid acts as an antihemolytic agent. In the case of obstructive jaundice it appears that the increase in the cholesterol content of the blood fluid is responsible for the great increase in resistance of the red cells against sapotoxin solutions.

TABLE VI  
JAUNDICE AND RED CELL RESISTANCE TO SAPOTOXIN SOLUTIONS

TYPE	CASES	RESISTANCE	1:13,000	Hb.	VARIATION IN RESISTANCE
Normals	185	1:13,037	10.7	91	-----
All Types	31	1:12,685	55.0	79	+ 1,252
Obstructive	15	1:10,033	?	84	+ 3,904
Hemolytic	5	1:18,600	6.8	81	- 4,663
General	11	1:13,182	?		+ 755
Cirrhosis	4	1:14,500	15.8	77	- 563

TABLE VII  
RELATION OF THE BLOOD FLUIDS TO RESISTANCE OF THE RED CELLS

EXP. NO.	RESISTANCE OF CELLS IN WHOLE BLOOD	WASHED CORPUSCLES			CHOLESTEROL
		DILUTED 1:1 WITH NaCl SOLUTION	DILUTED 1:1 WITH OWN SERUM	DILUTED 1:1 WITH SERUM OF OPPOSITE BLOOD	
X.	1:12,750	1:38,000	1:13,750	1:16,750	166
Y.	1: 9,000	1:34,000	1:11,500	1:17,500	292
Difference	3,750	4,000	2,250	6,750	126

Case X was suffering from a slight attack of gastritis.

Case Y was suffering from a severe attack of obstructive jaundice.

#### VIII. ANEMIA AND THE RESISTANCE OF THE RED BLOOD CELLS

The lowered resistance of the red blood cells in pernicious anemia has been appreciated for a long time. In other forms of anemia conflicting reports have appeared relative to the red cell resistance. In secondary anemias the resistance to salt solutions is raised. "Anemia" in itself means only that the number of red cells per given volume or their hemoglobin content is decreased. The causes of anemia are many, hence, the factors giving rise to this condition are of more importance from the clinical standpoint than the mere reduction in the cell count or the degree of hemoglobin. Anemias are associated with many diseases and their causative factors, it seems, should determine the blood cell reactions to hemolytic agents. Furthermore, the chemistry of the blood in cases of anemia determine the degree of resistance to laking agents, for the red cells must of necessity remain approximately

normal in order to exist as cells. Also, a process of cell destruction is continually at work in the normal blood stream, hence alterations in the blood chemistry may permit the hemolytic agents normally present unopposed play upon the red cells resulting in their untimely destruction. Oleic acid in excess in the blood stream acts as a powerful hemolytic agent. A continued action of such an acid must of necessity result in an anemia.

The work on the cholesterol content of the blood seems to throw some light on the probable cause of the various degrees of resistance shown by the red cells in different types of anemia. In pernicious anemia there is a definite reduction in the cholesterol content of the blood. Dennis<sup>12</sup> reports that the removal of the spleen results in an increased cholesterol content of the blood in every case, but that there is no change in the fat content of the blood. The excellent works of Bloor<sup>3</sup> have shown that the lipid value of the blood in anemia is normal as long as the percentage of blood corpuscles remains above one-half the normal number per volume. Whenever the percentage drops below this level abnormalities appear, which in the order of their magnitude and occurrence are: (1) high fat in plasma; (2) low cholesterol in plasma and occasionally in the corpuscles; (3) low lecithin in the plasma. These results, it is true, offer no certain evidence that abnormalities in the blood lipids are responsible for anemia, however, the low value for cholesterol which is an antihemolytic substance, and the high fat fraction which may indicate the presence of abnormal amounts of hemolytic lipids in the blood may be considered as possible causative factors in the production of anemia. Such argument is supported by the increase in cholesterol of the blood following splenectomy in cases of anemia.

Inasmuch as cholesterol is known to act as an antihemolytic agent against sapotoxin, the varying degrees of resistance offered by the red blood cells in cases of anemia may be assumed to depend upon the degree of protection offered by the serum to the cells during the presence of a hemolytic agent. Such a suggestion seems probable in the light of our present results.

Cases showing a reduction in the red cell count demonstrated varying degrees of resistance to laking agents. The average resistance of 43 such cases demonstrated a resistance to sapotoxin of 1:13,889, practically normal. The average red cell count was 3,022,553. The average hemoglobin was 55 per cent.

TABLE VIII  
ANEMIA AND THE RESISTANCE OF THE RED BLOOD CELLS TO SAPOTOXIN

ANEMIA	CASES	RESISTANCE	1:13,000	Hb.	RED COUNT
Pernicious	8	1:15,063	8.0	35	1,135,000
Cardiorenal	8	1:12,312	8.5	60	2,570,875
Syphilis	7	1:15,750	10.4	58	3,936,000
Hemolytic					
Jaundice	4	1:17,125	7.7	58	3,050,000
Carcinoma	5	1:12,190	17.3	51	2,796,000
Tuberculosis	4	1:13,250	13.4	60	4,263,233
Undetermined	7	1:13,040	16.0	58	3,496,666
All cases	43	1:13,889		55	3,022,553
Normals	185	1:13,937	10.7	91	Not determined

Anemias seem to divide into three types, those with a high resistance, those with practically normal resistance and those with low resistance (Table VIII).

The average resistance of 8 cases of pernicious anemia was 1:15,063, or a reduction from the average normal of 1,126 points. Eight cases of anemia associated with cardiorenal diseases gave an average of 1:12,312; a resistance 2,751 points greater than for pernicious anemia. The average resistance of 7 luetic cases was 1:15,750 or a reduction of 1,813 points below the average normal. Four cases of hemolytic jaundice with low red cell counts gave an average resistance to sapotoxin of 1:17,125 or a reduction of 3,188 points below the average normal. Five carcinoma cases with low cell counts gave an average resistance of 1:12,190 or an increased resistance of 1,747 points over the average normal. Tuberculous patients (4) showing low cell counts gave an average resistance to sapotoxin solutions of 1:13,250 or 687 points greater than the normal resistance. The remaining 9 cases showed a reduction in the red cell count from undetermined and various causes. The average resistance for these cases was 1:13,040 or an increase over the average normal of 897 points.

#### IX. CARDIORENAL DISTURBANCES AND RED CELL RESISTANCE

Arterial hypertension is often accompanied by an increased resistance of the red cells. A similar increased resistance has also been reported for nephritis and diabetes. In eclampsia the resistance is markedly increased to anisotonic salt solutions and to saponins.

Cholesterol remains normal or is increased in nephritis (Gorham<sup>13</sup>), however, cholesterol combined as esters is low. Bloor<sup>5</sup> has found that in severe nephritis there is a high content of fat in the plasma and corpuscles, and an increased amount of lecithin in the corpuscles. The cholesterol content remains essentially normal. Similar conditions are found in alimentary lipemia. Inasmuch as an alkalinity of the blood and tissues is necessary for their normal functioning, it seems probable that the relation of fat assimilation found in nephritis is one manifestation of a general phenomenon brought about by a decreased blood and tissue alkalinity. The observations of De Langen are of interest.<sup>11</sup> He reports that the Javanese peoples show about one half the cholesterol value of the blood as do the Europeans. Gall bladder trouble, diabetes and chronic nephritis is rare among these peoples.

Twelve cases of nephritis gave an average resistance of 1:13,958 (Table IX). The average hemoglobin was 79 per cent. The average findings for chemical analyses of the blood of these patients was urea nitrogen 76.8, uric acid 6.21, creatinin 5.30, sugar 0.127 and carbon dioxide 30.7.

Five cardionephritic cases gave an average resistance of 1:14,500 or a reduction of 563 points below the normal average. The chemical analysis of the blood of these patients was urea nitrogen 24.5, uric acid 3.50, creatinin 1.82, sugar 0.156 and carbon dioxide 55.0.

Two cases of diabetic coma averaged a resistance of 1:13,500. The hemoglobin was 88 per cent and blood sugar 1,700.



TABLE IX  
KIDNEY DISEASES AND THE RESISTANCE OF THE RED CELLS TO SAPOTOXIN

DISEASE	CASES	RESISTANCE	1:13,000	Hb.	RESISTANCE VARIATION
Nephritis	12	1:13,958	17.5	79	Normal
Cardiomephritis	5	1:14,500	10.3	84	- 563 points
Eclampsia	2	1:10,500	20.4	83	- 3,437 points
Uremia	5	1:14,050	16.7	85	Normal
Diabetes	2	1:13,500	14.0	88	- 437 points
Calcium Chloride*	15	1:13,950	--	90	Normal
Ammonium Chloride*	15	1:13,317	--	90	- 620 points
Urea*	6	1:12,666	24.6	90	- 1,271 points
Normals	185	1:13,937	19.7	91	-----

\*Average of all determinations while on drug.

Five cases of uremia gave an average resistance of 1:14,050. The hemoglobin averaged 85 per cent. Chemical analyses of the blood showed a urea nitrogen content of 16.5 and carbon dioxide of 58.

Two cases of eclampsia demonstrated a resistance of 1:10,500 or an increase in the red cell resistance of 3,437 points above the average normal.

The addition of 1 to 3 minims of a 1 per cent solution of urea causes the resistance of normal red cells to become increased. Three determinations on the normal blood gave an average resistance of 1:14,000, hemoglobin 90; time for complete hemolysis to occur in a 1:13,000 solution 12 minutes. The average resistance of 6 determinations to which urea solutions had been applied was a resistance of 1:12,666; complete hemolysis 24.6 minutes; hemoglobin 90 per cent.

The effect of calcium and ammonium chloride upon red cell resistance was determined in 15 cases each (Table IX). The average resistance of the red cells in the calcium experiment was 1:13,950. The average hemoglobin was 90. The first day following the taking of 60 grains of calcium chloride the resistance was 1:13,300; the second day 1:13,700 and the third day 1:14,100. Calcium chloride, therefore has little or no effect upon the resistance of the red cells to sapotoxin solutions. The average of 15 cases was 1:12,900. The first day following the ingestion of 45 grains of ammonium chloride resulted in an increased resistance of 1,037 points above the normal average. The second day the resistance was 1:13,000, an increase of 937 points and the third, 1:14,900. Therefore, ammonium chloride causes an increase in the resistance of the red blood cells immediately following its administration, however, resistance is not permanently influenced by ammonia, for on the third day the resistance is back to normal.

#### X. MALIGNANT GROWTHS AND THE RESISTANCE OF THE RED BLOOD CELLS

The resistance of the red cells may or may not be reduced in cases of malignancy.

Gorham and Myers<sup>12</sup> state that a condition of hypocholesterolemia is found in the cachexia of malignancy and all anemias of the pernicious type. Bloor and Knudson<sup>3</sup> have shown that in normal blood there is a constant re-

lation between the free cholesterol and cholesterol esters. In whole blood the average percentage of cholesterol in combination as esters is about 33.5 per cent, in plasma 58 per cent of the total cholesterol. In pathologic conditions the relation between free and bound cholesterol remains normal in all cases except carcinoma and most cases of nephritis, in both of which the per cent combined as esters is low. Luden<sup>14</sup> has shown that the cholesterol content of the blood may be increased by diet also that a cholesterol increase results in a coincident weakening of the lymphoid defense. Hence, a decrease in the lymphoid defense accompanied by an increase in cholesterol may act as a predisposing factor in the production of cancer.

The resistance of red blood cells in 19 cases of malignant growths of various parts of the body gave an average resistance of 1:13,079. This represents an increased resistance of 858 points above the normal average. The average time required for complete laking in a 1:13,000 solution was 16 minutes. The greatest individual resistance (Case No. 890) 1:9,000 was found associated with a carcinomatous gall bladder. The lowest resistance—1:14,500—(Case No. 537) was shown by one suffering from visceral growths and syphilis. The average hemoglobin of the 19 cases studied was 78 per cent. Hence, there

TABLE X  
MALIGNANT GROWTHS AND RED CELL RESISTANCE

	CASES	RESISTANCE	1:13,000	HB.
	19	1:13,079	16.0	78
Normals	185	1:13,937	10.7	91
Difference		+ 858	5.3	13

is a slight increase in the resistance of the red blood cells in cases of malignant growths. However there is no constancy in the determinations; other pathologic conditions greatly alter the results. However, the early cases of carcinoma did show a higher degree of resistance in the red cells than in those cases associated with anemia or marked cachexia (Table X).

#### SUMMARY AND CONCLUSIONS

*I. Pregnancy* is associated with an increased resistance of the red cells to sapotoxin. At the time of delivery the blood cells show their greatest or maximal resistance to the action of sapotoxin. Immediately following parturition the resistance of the erythrocytes begins to diminish and upon the 12th day has materially returned toward the normal. Hemoglobin determinations vary but slightly during the latter part of pregnancy and immediately following parturition. The increase in blood cholesterol, may in part account for the rise in red cell resistance to sapotoxin.

*II. Malaria* as such does not appear to lower the resistance of the red blood corpuscles to sapotoxin. The administration of quinine causes a decrease in the resistance, this decrease lasting throughout the period of quinine administration. Nonmalarial cases fail to show any constant deviation from the normal average resistance either during the taking of quinine or following its withdrawal. Hence the loss of resistance on the part of malarial blood following the administration of quinine is characteristic of malaria plus quinine.

That is, quinine appears to act in such a manner as to bring about a weakened resistance in malarial cells. Therefore the assumption may be made that quinine aids in the destruction of the red cells carrying the plasmodium of malaria. The low cholesterol content of the blood also predisposes to the hemolytic substances present during malaria.

*III. Pulmonary Tuberculosis* tends to increase the resistance of the red blood cells to the hemolytic action of sapotoxin. Pregnancy in such cases accentuates the effects of tuberculosis. Tuberculosis complicated with syphilis tends to bring about a depression in the resistance of the red cells.

*IV. Typhoid Fever* appears to alter but little the resistance of the red cells.

*V. In Syphilis*—positive Wassermann—the resistance of the red cells is less than that for the average normal. The administration of mercury alone tends to increase the resistance. The addition of iodides to the mercurial treatment causes a reduction in the resistance of the red cells for several days; the effects cannot be maintained. Luetic patients on iodides alone show but little change in the resistance of the red cells.

*VI. Lead Poisoning* may bring about an increased resistance of the red cells to sapotoxin solutions.

*VII. Jaundice* and blood cell resistance appear to bear no constant relationship, that is, jaundice as such is in no way an indication of blood cell resistance. Obstructive jaundice causes a marked increase in the resistance of the blood cells, on the other hand, hemolytic jaundice is associated with a marked decrease in the resistance of the erythrocytes. Other types of jaundice may or may not be associated with variations in the resistance of the red cells. The relation of blood cholesterol to red cell resistance is such as to lead to the assumption that cholesterol is a depressant to the hemolytic action of sapotoxin.

*VIII. Anemia* associated with lues and hemolytic jaundice shows a lessened degree of resistance of the red cells to sapotoxin. The red cells in anemias associated with or caused by carcinoma, tuberculosis, obstructive jaundice and some cases of pernicious anemia show an increased resistance towards hemolytic agents. Anemia as such does not appear to affect the red cell resistance, something more than a mere reduction in the number of cells determines the degree of the resisting power of the red blood cells.

*IX. Cardiorrenal Diseases.* Arteriosclerosis is associated usually with an increased resistance of the red cells to sapotoxin. Diabetic coma and uremic conditions in general are not associated with marked alterations of the red cell resistance. Cases diagnosed as nephritis give normal resistance reactions, however, cases diagnosed as cardionephritics seem to show some slight degree of reduction of resistance. The addition of urea solutions to red cells tends to increase their resistance. Calcium chloride administered by mouth does not affect the red cell resistance. The taking of ammonium chloride by mouth tends to increase the resistance of the cells for a short period only. The resistance returns to normal even during the administration of the drug. The relation of cholesterol in the blood in cardionephritic cases to the resistance of the red cells is striking. The increased lecithin in the corpuscles of severe nephritis, however, leads one to suspicion the validity of this relation. How-

ever, the increased cholesterol of the serum may be sufficient to protect the cells in spite of the increased lecithin content.

*X. Malignant Growths* as such do not appear to alter the red cell resistance; there is no constancy in the determinations. However, the early cases of carcinoma show a slightly higher resistance than cases associated with anemia or cachexia.

*XI. The Cholesterol* content of the blood appears to run parallel to the degree of resistance of the red cells to sapotoxin solutions. A high cholesterol content of the blood is associated with a high degree of resistance of the red cells when they are surrounded with serum. A low cholesterol content is associated with a reduced resistance of the cells.

*XII.* Therefore, the resistance of the red cells to sapotoxin solutions varies in different diseases and this variation is intimately associated with alterations in the metabolism of cholesterol.

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# LABORATORY METHODS

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## A SYSTEM OF LABORATORY EXAMINATIONS AND RECORDS\*

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THE following system of making and filing requests and reports for laboratory examinations has been gradually constructed and found uniformly satisfactory in the wards and dispensaries of the Polyclinic and Medico-Chirurgical hospitals of the Graduate School of Medicine of the University of Pennsylvania.

The problem of constructing an efficient system is frequently troublesome and probably no single system will meet all conditions in individual institutions; the essential requirements which I have had in mind in piecing together the present system have been as follows:

1. A means for making requests for laboratory examinations in writing with the minimum of time and work. This condition has been met by adopting a request card devised by Dr. C. Y. White, which the physician can fill out in a few minutes.

2. A means for furnishing the laboratory with some clinical data in the interests of more intelligent work and cooperation. This condition is fulfilled by the same request card.

3. A means for returning a report to the hospital or out-patient clinic and making a duplicate for filing in the laboratory with the minimum of time and work.

4. A means for recording the results of laboratory examinations on the history of each patient in such manner as permits binding and avoids too much bulk.

5. A system for filing and recording duplicate reports in the laboratory readily accessible and facilitating the compilation of weekly, monthly or yearly reports with a minimum of work and trouble.

6. A means for providing each physician with a record of laboratory examinations which he may use for his private records.

The system herein described has met these essential requirements and can be readily modified if necessary to suit individual conditions.

### THE REQUEST CARD

In most institutions requests for laboratory examinations are made on the upper half of printed blanks of paper of the size of history sheets which are filled out with the name of the patient, ward and such essential data and for-

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\*From the Department of Medical Sciences of the Medico-Chirurgical Graduate School of Medicine of the University of Pennsylvania.



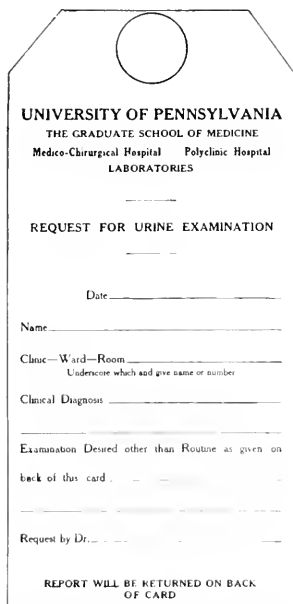
[illegible]

Fig. 2. Back of request card

warded to the laboratory with the specimen; the laboratory report is written on the lower half of the blank and the whole sheet returned to the hospital.

The usual objections to this system are: (a) the sheet may become soiled and creased; (b) when a large number of examinations are made an accumulation of these blanks renders the history too bulky for binding and (c) these blanks usually fail to furnish the pathologist with some clinical data. This system is particularly apt to be unsatisfactory in out-patient departments unless the laboratory reports are transcribed to the history card.

The request blank shown in Figs. 1 and 2 is slightly modified from that



**UNIVERSITY OF PENNSYLVANIA**  
**THE GRADUATE SCHOOL OF MEDICINE**  
**Medico-Chirurgical Hospital Polyclinic Hospital**  
**LABORATORIES**

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**REQUEST FOR URINE EXAMINATION**

-----

Date \_\_\_\_\_

Name \_\_\_\_\_

Clinic—Ward—Room \_\_\_\_\_  
Underline which and give name or number

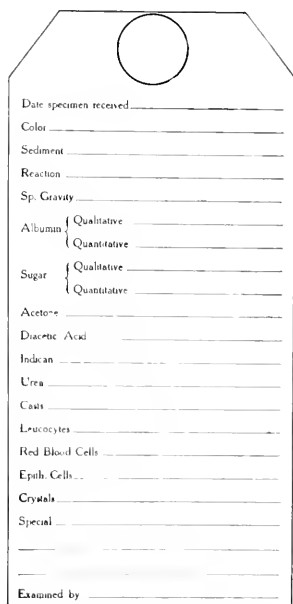
Clinical Diagnosis \_\_\_\_\_

Examination Desired other than Routine as given on  
 back of this card \_\_\_\_\_

Request by Dr. \_\_\_\_\_

**REPORT WILL BE RETURNED ON BACK  
 OF CARD**

Fig. 3. Front of card used for routine urine examinations.



Date specimen received \_\_\_\_\_

Color \_\_\_\_\_

Sediment \_\_\_\_\_

Reaction \_\_\_\_\_

Sp. Gravity \_\_\_\_\_

Albumin { Qualitative \_\_\_\_\_  
 { Quantitative \_\_\_\_\_

Sugar { Qualitative \_\_\_\_\_  
 { Quantitative \_\_\_\_\_

Acetone \_\_\_\_\_

Diacetic Acid \_\_\_\_\_

Indican \_\_\_\_\_

Urea \_\_\_\_\_

Casts \_\_\_\_\_

Leucocytes \_\_\_\_\_

Red Blood Cells \_\_\_\_\_

Epih. Cells \_\_\_\_\_

Cystals \_\_\_\_\_

Special \_\_\_\_\_

Examined by \_\_\_\_\_

Fig. 4. Back of card used for routine urine examinations.

devised by Dr. C. Y. White of Philadelphia and used in the Episcopal hospital; it has been found very serviceable and satisfactory.

The blank is printed on thin cardboard, measures 4 by 12 inches and is perforated in the middle so that it may be divided into two cards.

Fig. 1 shows the front and Fig. 2 the back of this card.

The physician fills out the left-hand portion of the card (Fig. 1) which provides space for the name of the patient, age, ward, date and name of attending physician; simply drawing a line under the nature of the specimen and the kind or kinds of examinations requested and signing completes the essentials. Space is provided for the clinical diagnosis (usually provisional)



and also for further details of the examination requested, if the latter is necessary. Space is also provided for the chief clinical data bearing upon the laboratory examination; for example, the kind and duration of treatment in relation to a request for the Wassermann test. *Only the first request card for a patient need be filled out with the details of diagnosis and clinical data*; when subsequent examinations are requested the essential data alone is sufficient (name of patient and physician, date, ward and examination requested). Dr. White uses a green card for the first request and white cards for subsequent requests. I have found this a good scheme but likely to produce confusion and a great deal of trouble in insisting upon the proper use of the two cards; for these reasons I have abandoned the use of the green card as being nonessential.

A clerk in the laboratory records on each half of the card the date and hour received and subsequently the date when the report is returned (which are frequently the means for settling a dispute regarding a delay in returning a report due to delay in forwarding the request or specimen or both).

UNIVERSITY OF PENNSYLVANIA  
Graduate School of Medicine  
Medico-Chirurgical Hospital      Phys. and H. Sp. Hall

**IDENTIFICATION SLIP**

To be filled out and attached to all containers with specimens of blood, pus, cultures, tissues and cerebrospinal fluid. **Do not use** for specimens of urine

Date \_\_\_\_\_

Patient's Name \_\_\_\_\_

Clinic-Ward-Room \_\_\_\_\_  
(Laboratory and H. Sp. Hall)

Sent by \_\_\_\_\_

This is not a request for examination, send regular request card

Fig. 5.—Front of the identification tag attached to specimens.

**Paste this label securely on container. If necessary use a small piece of adhesive tape.**

**Send to laboratory without delay together with request card.**

Fig. 6.—Back of the identification tag attached to specimens.

The right-hand half of the card is filled out in the laboratory although some physicians are willing to give an extra minute or two for this: *the laboratory report is returned to the hospital on this half of the card*. The pathologist also writes a synopsis of his report on the left-hand portion of the card.

*The left-hand half of the card is filed in the laboratory (alphabetically); the right-hand half is returned to the hospital.* A cross index may be kept in the laboratory for filing the necropsies, examination of tissues, blood, sputa, etc.

The results of blood examinations are filled in on the back of each card, which also provides additional space for other laboratory reports.

*As a general rule only one kind of an examination is requested on a card.* For example, a general blood examination including erythrocyte and leucocyte counts, differential leucocyte count, hemoglobin estimation and color index may be requested on one card but it is not permissible in these laboratories to request on one card for example, a general blood examination and an examination of sputum or some other examination; a second card would be required

for an examination of the sputum. The main reasons for this are to prevent overcrowding a card with the necessary laboratory reports and to facilitate filing a laboratory record.

*This card is not used for requests for routine urine examinations; a special card is provided shown in Figs. 3 and 4, which is tied to the bottle or container and the report returned on the back of the same card (Fig. 4).*

Dr. John Eiman, pathologist to the Presbyterian Hospital of Philadelphia, has suggested the use of rubber stamps in the laboratory for stamping the cards in making out reports; I use the following list and find them very useful for saving time and overcoming the difficulties of poor and illegible writing:

BLOOD EXAMINATIONS	
Anisocytosis	Polychromatophilia
Poikilocytes	Basophilic degen.
Macrocytes	Myelocytes
Microcytes	Coagulation Time
Macroblasts	Malaria
Normoblasts	Platelets
Microblasts	Culture
GASTRIC ANALYSIS	
Amount	Total Acidity
Appearance	Free HCl
Odor	Comb. HCl
Mucns	Acid Salts
Blood	Lactic Acid
Microscopical:	Fractional Analysis:
	Total Acidity
	Free HCl
	Protein
PUNCTURE FLUIDS	
Amount	Color
Appearance	Specific Gravity
Reaction	Sugar
Proteins	
Cells per 1 c. mm.	
Polys	% Lymph
R. B. C.	% Endoth
Smears and Cultures	Animal Inoculation
Colloidal Gold	
FECES EXAMINATION	
Form	Bile
Consistency	Reaction
Color	Curds
Mucns	Concretions
Blood	Parasites
Pus	Ova
Microscopical:	Bacteriological:
SPUTUM EXAMINATIONS	
Quantity	Blood and Pus
Appearance	Tubercle Bacilli
Color	Fungi
Cells	Pneumococci Type
BACTERIOLOGICAL	
Smear of	Result
Culture of	Result
WIDAL REACTION	
Time	
Dilutions	
Results	

Specimens of tissue, feces, sputa, etc., may be labeled with adhesive bands bearing the name of the patient; I have used the identification tag shown in Figs. 5 and 6 with entire satisfaction.

In this institution the specimens and cards are carried by the nurses or orderlies to a specimen room set aside in each hospital; at 9 and 11 A. M. and 3 P. M. a laboratory orderly collects these and brings them to the laboratories. This system prevents confusion and tends to fix responsibility for delivery of request cards and specimens.

*Urgent examinations are made at any time, the physician marking the card "rush" and sending it at once to the laboratories by a nurse or orderly; these requests receive instant attention.*

In exceptional instances telephone requests are accepted *but are always followed up by the request card properly filled in* with the necessary data.

Requests for necropsies are made out on the same card (the legal permits being kept and filed in the office of the hospital); necropsy reports are returned on a set of blanks modified and enlarged by Dr. White from those originally devised by the late Dr. A. O. J. Kelly. These printed blanks are unusually complete and when properly filled out furnish a splendid record of a necropsy which is easily bound with the clinical history.

All laboratory examinations are made as far as possible on the same day as when the requests and specimens are received; the reports are returned to the respective wards and out-patient departments of the hospitals once or twice daily by the resident physicians serving in the laboratories, this system definitely fixing responsibility for the prompt and safe delivery of reports.

#### RECORDING LABORATORY REPORTS ON HISTORY

When the reports are returned to the hospital they are copied by the resident physicians, nurses, or a special clerk to the histories of the patients. The necessity for copying constitutes the single weak point in this system by reason of the opportunity for error in copying the reports or individual carelessness. As a matter of experience, however, errors rarely occur and none have occurred since rubber stamps have been used as described above in making out laboratory reports, which largely eliminates bad writing.

Three blanks are provided for these reports shown in Figs. 7, 8 and 9; each sheet measures 9½ x 12 inches and is bound with the rest of the history.

The first sheet (Fig. 7) provides space for recording seven routine and three special examinations of blood and urine. If only urine and blood examinations have been requested, this would be the only sheet required.

The second sheet (Fig. 8) provides for recording examinations of stomach contents and vomitus, feces, sputum and serological reactions. This sheet is attached to the history only in case any of these examinations have been made in order to avoid the waste of binding a blank.

The third sheet (Fig. 9) provides space for recording the results of examinations of cerebrospinal fluid, pus, transudates, cyst fluids and secretions; also general bacteriological examinations, examination of tissues and special chemical examinations of blood and urine.

## UNIVERSITY OF PENNSYLVANIA

The Graduate School of Medicine

Medico-Chirurgical Hospital

Polyclinic Hospital

Name \_\_\_\_\_

Case No. \_\_\_\_\_

## LABORATORY REPORTS (1)

## GENERAL URINE EXAMINATIONS

DATE						
Quantity						
Color						
Sediment						
Reaction						
Sp. Gravity						
Albumin (Qualitative)						
Albumin (Quantitative)						
Sugar (Qualitative)						
Sugar (Quantitative)						
Acetone						
Diacetic Acid						
Butyrobutyric						
Indican						
Urea						
Bile						
Total Nitrogen						
Microscopical						
Cells						
Leucocytes and Pus						
Red Blood Corpuscles						
Epith. Cells						
Cylinders						
Mucus						
Crystals						
Special						
Examiner						

## SPECIAL URINE EXAMINATIONS

DATE			
Functional Kidney Test			
Bacteriological			
Animal Inoculation for T. B.			
Special Chemical Exam.			
Examiner			

## GENERAL BLOOD EXAMINATIONS

DATE						
Erythrocytes						
Leucocytes						
Hemoglobin						
Color Index						
Differential						
Small Lymph						
Large Lymph						
Transferricals						
Polymorph.						
Eosinophiles						
Basophiles						
Abnormal Erythrocytes						
Abnormal Leucocytes						
Examiner						

## SPECIAL BLOOD EXAMINATIONS

DATE			
Blood Culture			
Coagulation Time			
Malaria			
Basophilic Degeneration			
Blood Platelets			
Parasites			
Special Chemical (Urea, Sugar, etc.)			
Examiner			

Fig. 7. Sheet used for recording results of laboratory examinations; bound with the history.

## UNIVERSITY OF PENNSYLVANIA

*The Graduate School of Medicine*

Medico-Chirurgical Hospital

Polyclinic Hospital

Name \_\_\_\_\_

Case No. \_\_\_\_\_

## LABORATORY REPORTS (2)

## SEROLOGICAL EXAMINATIONS

DATE			
Wassermann			
Geno. Compl. Fixation			
Widal			
Special			
Examiner			

## GASTRIC ANALYSIS (INCLUDING VOMITUS)

DATE			
Amount			
Gross Appearance			
Odor			
Total Acidity			
Free Hydrochloric Acid			
Combined Acids			
Acid Salts			
Lactic Acid			
Blood			
Ferments			
Fractional Analysis			
Total Acidity			
Free Hydrochloric Acid			
Protein			
Microscopical Examination			
Special			
Examiner			

## GENERAL FECES EXAMINATIONS

DATE			
Form			
Consistency			
Color			
Mucus			
Blood			
Pus			
Bile			
Reaction			
Curds			
Coagulations			
Parasites			
Ova			
Microscopical			
Special			
Examiner			

## SPECIAL FECES EXAMINATIONS

DATE			
Cultures—Bacteriological			
Animal Inoculation for T. B.			
Examiner			

## SPUTUM EXAMINATIONS

DATE				
Quantity				
Character—Gross Appearance				
Color				
Microscopical				
Cells				
Fungi				
Blood and Pus				
Bacteriological				
Tubercle Bacilli				
Pneumococci—Type				
Special				
Examiner				

Fig. 8.—Sheet used for recording results of laboratory examinations; bound with the history.

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*The Graduate School of Medicine*

Medico-Chirurgical Hospital

Polyclinic Hospital

Name.....

Case No. ....

## LABORATORY REPORTS (3)

## CEREBROSPINAL FLUID, PUS, TRANSUDATES, CYST FLUID AND SECRECTIONS

DATE				
Amount				
Gross Appearance				
Color				
Sp. Gravity				
Reaction				
Pressure				
Cells per Cubic Millimeter				
Kinds of Cells				
Lymphocytes				
Endothelial				
Polymorphonuclears				
Red Blood Corpuscles				
Protein				
Sugar				
Smears				
Cultures				
Animal Inoculation				
Wassermann Reaction				
Colloidal Gold Reaction				
Special				
Examiner				

## GENERAL BACTERIOLOGICAL EXAMINATIONS

DATE				
Vaginal Smears and Washings				
Smears (mention source)				
Cultures of Throat and Nose				
Cultures of Other Parts (mention)				
Special				
Examiner				

## EXAMINATION OF TISSUES REMOVED AT OPERATION

DATE				EXAMINER

## SPECIAL CHEMICAL EXAMINATIONS OF BLOOD AND URINE

DATE				
Sugar				
Creatinine				
Creatine				
Uric Acid				
Urea				
Non Protein Nitrogen				
Chlorides				
Carbon Dioxide Combining Power				
Total Nitrogen				
Total Solids				
Examiner				

Fig. 9. Sheet used for recording results of laboratory examinations; bound with the history.

Similar blanks are used for recording laboratory reports in the out-patient departments inasmuch as the history blanks are of the same size as used in the hospitals.

#### PROVIDING SEPARATE REPORTS FOR THE ATTENDING PHYSICIAN

When a report has been copied from a card to the history as described, the latter has fulfilled its function and may be discarded. It is marked "received and copied" and attached temporarily to the history board where the attending physician may obtain it, "post-officed" in the physicians' room of the hospital or mailed direct to the physician; by any of these methods the physician may obtain the report for his private records.

This is a matter of considerable importance and convenience when the patient leaves the hospital before the laboratory examinations are completed and the reports returned; under these circumstances the reports are taken to the office of the hospital and copied on the history but the physician may never see the report. With any of the above plans in operation the attending physician is bound to receive the report and experience has taught that *no system is successful unless it literally forces the report upon the attention of the physician with the minimum of effort on the part of the latter.*

#### LABORATORY RECORDS

When a request card is received in the laboratory by a clerk it is recorded, given a serial number and the date and hour written on both halves of the card (upper right-hand corners) as shown in Fig. 1. *All request cards and specimens are delivered to a single designated place in the laboratory so that all pass through the hands of a clerk for purposes of record.* Even "rush" requests are first recorded, although in case of unavoidable delay in recording the card the examination may proceed and the record be made afterward.

The main purpose of recording each request card is to guard against loss and provide a means for tracing a card in case of a miscarriage. For example, a request card may miscarry in the laboratory after being recorded.

For the purpose of recording the requests for laboratory examinations, seven cards of *different colors* are employed. Each card measures six by nine inches, is ruled on both sides and provides from forty to fifty entries.

Fig. 10 shows the card used for recording requests for blood examinations; this card is colored red.

Fig. 11 shows the card for feces examinations (brown).

Fig. 12 is the card for examination of stomach contents and vomitus (pea green).

Fig. 13 is the card for sputum examinations (gray).

Fig. 14 is the card for examinations of cerebrospinal fluid, pus, transudates, cyst fluid and secretions (white).

Fig. 15 is the card for general bacteriologic examinations (yellow).

Fig. 16 is for recording requests for tissue examinations and necropsies (pink).

Fig. 17 is the card for recording special urine examinations (yellow). Routine urine examinations are not recorded separately; simply the total number





*The number of any kind of examination or examinations can be seen at a glance and at the end of a week, month or year a detailed laboratory report or summary can be prepared by a clerk with a minimum of time and work by simply summarizing these cards. The system has proved eminently satisfactory.*

RECORD OF STOMACH CONTENTS AND VOMITUS EXAMINATIONS

[illegible]

Fig. 12.—Card (pea green) used for filing requests for examination of stomach contents.

RECORD OF SPUTUM EXAMINATIONS

[illegible]

Fig. 13.—Card (gray) used for filing requests for sputum examinations.





## A MODIFICATION OF THE TECHNIC OF THE VIVIDIFFUSION METHOD OF ABEL\*

BY H. C. VAN DER HEYDE AND WITHEROW MORSE, MORGANTOWN, W. VA.

THE principal difficulty encountered in the vividiffusion method first suggested by Abel seems to be the trouble encountered in the preparation of the dialyzing tubes, as one may judge from the several suggestions which have been made to simplify this procedure.

In this laboratory, we find that by following the method outlined here, it is possible to save much time and patience.

A glass rod of a size slightly larger than the diameter of the tubes within the apparatus is dipped about a decimeter into a fairly liquid collodion and about this rod is wrapped lengthwise, a piece of fish bladder tissue about 12 cm. long and 1.5 cm. wide, permitting an extension of about two centimeters of the tissue beyond the end of the glass rod, so that one may remove the cylinder by pulling upon this extension. The cylinder is slipped off the rod before the collodion has set to such an extent that it will cause the tissue to adhere to the rod. The cylinders may then be dried by clamping them in a vertical position, or by pinning them to the sides of the table. If it is desired to limit the collodion as much as possible, the rod may be bathed with a somewhat more viscous collodion down one side and the tissue wrapped so that only the overlapping edge comes into contact with the collodion. It generally happens with this method, that one must apply a line of collodion down the seam to insure perfect adhesion.

Hess and McGuigan (*Jour. Pharmacol. and Exper. Therap.*, 1914, vi, 1) modified Abel's original technic (*Jour. Pharmacol. and Exper. Therap.*, 1914, iv, 611) and more recently, Love (*Med. Rec.* 1920, xcviii, 649) has advocated the use of chicken intestine for the purpose of replacing the collodion tubes. We are under the impression that the modification suggested above may make the application of this valuable method somewhat easier.

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## EDITORIALS

### *Present-Day Methods for Studying the Problem of Ventilation*

INVESTIGATION of the problem of ventilation is greatly hampered by the fact that of all animals man alone is concerned. This limits the possibilities for the investigation of the problem which, in brief, consists in comparing the general well being and comfort of man with the physical conditions of the indoor atmosphere in which he is living.

In a previous editorial in this journal,<sup>1</sup> the older theories of ventilation were reviewed and it was shown that neither the chemical composition of the air nor the presence in it of organic poisons has anything to do with the unhealthful effects which are associated with living in inadequately ventilated places. The conclusion was arrived at that it is the cooling effect of the air on the body which determines its healthfulness. The body is constantly producing heat by the metabolic processes which go on in it. This heat must be lost as quickly as it is produced, else will the temperature of the blood rise and the secondary reactions of a mild fever be the result. A great part of this heat loss occurs at the surface of the body by physical factors which depend on the cooling effect of the air. Of these factors radiation, convection and

evaporation are the most important although the last mentioned of these only comes into play to any considerable extent at the surface of the body when the air temperature is above a certain level which is somewhere about 70° F. There is, of course, some heat constantly lost by evaporation of water in the lungs but in man this is relatively constant (provided the absolute humidity and the temperature of the air remain unchanged), and it is the loss of heat from the skin by radiation and convection which is subject to alteration by changes in the blood flow in the superficial vessels. By observations on rabbits, N. B. Taylor and I have found by the use of thermocouples that there may be a difference of several degrees of temperature between the deeper tissues and those that immediately underlie the skin. This indicates a great cooling effect at the surface of the body. When the blood vessels in the subdermal tissues become dilated, and a large proportion of the blood as a consequence becomes drafted to the surface, this cooling effect may become greatly increased and when constriction of these vessels occurs it may become very slight because of the low heat conductivity of the skin and subcutaneous fat.

In poorly ventilated places the cooling influence of the air becomes reduced for several reasons: first, the temperature of the air rises, thus diminishing radiation from the skin (which is dependent upon the difference of temperature between skin and air), as well as reducing the heat which is lost in the air passages in warming the inspired air; second, the air stagnates so that there are only feeble convection currents; third, the humidity rises (both relative and absolute), so that less heat is lost both in the respiratory passages (in saturating the expired air with water at body temperature), and at the skin. To compensate for the lesser cooling, the heat-controlling centers cause more blood to be sent to the surface of the body, by vasodilation of the cutaneous vessels, so that the skin becomes flushed, and this may become so marked as to cause throbbing in the head and other symptoms. This drafting of blood to the surface causes less to flow in the vessels of the viscera so that the digestive and other glandular functions are hampered and the brain is inadequately supplied, leading to drowsiness, etc. The mucous membranes of the upper respiratory passages also become swollen and congested, due largely to capillary dilation, and therefore more susceptible to infections. But the attempts to keep down the temperature are not restricted to readjustments of blood supply, for there is also a cutting down of the body furnaces by depression of the metabolic functions, and it is quite likely that incompletely metabolized substances accumulate in the tissues and organs and clog the wheels of life.

Probably a most important effect of the inefficient cooling is dependent on the fact that the rather steep temperature gradient which we have seen to exist normally between the surface and the deeper tissues of the body now disappears, with the consequence that the thermic nerve endings in the skin are no longer stimulated. The stimulation of these nerves depends not on the actual temperature of the skin, but on differences in temperature between the skin and the deeper tissues so that when the surface of the skin is at about the same temperature as the deeper structures they are not excited. The impulses set up by the stimulation of these thermic nerve endings are important in

maintaining the tone of the nerve centers. They furnish, along with visual and acoustic sensations, the most important afferent impulses to the nerve centers, to keep them in a "wakeful" state.

The practical problem in ventilation is therefore to find simple methods by which the cooling influence of the atmosphere may be measured. An ordinary thermometer is of little value since it merely records the temperature of the glass and wood of which the instrument is made. A wet bulb thermometer is somewhat more valuable for its reading, by comparison with that of the dry bulb, measures the extent to which evaporation of moisture into the air is capable of lowering the temperature of the surface of the instrument. Until quite recently, however, these were the only two physical instruments that could be employed to measure the cooling influence of the air, and consequently the efficiency of ventilation. A much more reliable instrument is the Kata thermometer of Leonard Hill.<sup>2</sup> This is an alcohol thermometer reading between 95° and 100° F. It is first of all placed in water at about 105° F., and when the alcohol has expanded to the full extent for that temperature, the thermometer is withdrawn and the time required for the meniscus to fall from 100° F. to 95° F., is measured by a stop watch. The above range of temperatures being about that of the body, it is clear that the cooling of the instrument will be proportional to the cooling at the surface of the body. To calculate the amount of heat which is actually lost, it is necessary to express the cooling power in heat units per square centimeter of surface per second. The unit of heat is a calorie (i. e., the amount of heat necessary to raise the temperature of 1 gm. of water through 1° C.), but for the present purpose one thousandth of a calorie is taken; that is, a millicalorie. In order to convert the reading in seconds into millicalories per square centimeter per second, all that is necessary is to divide seconds by a factor (the Kata factor), which is written on the stem of the instrument. When used with its surface dry, the cooling of the instrument is dependent upon convection and radiation, but it can be made to include the influence of evaporation as well by keeping the surface moist by covering the bulb with muslin (muslin glove finger) kept moistened with water.

Numerous readings have been taken by the Kata thermometer under all varieties of atmospheric conditions, both without and within doors, and the most significant feature of the results is that in the former case the readings vary greatly within short periods of time whereas indoors they are usually more or less constant. Even in relatively stagnant humid air, the fluctuations outside are in general much greater than inside, even in a fairly well-ventilated room. Leonard Hill in his report for the Medical Research Committee,<sup>2</sup> publishes numerous charts to illustrate these differences and also to show that the readings by the wet and dry Kata thermometer are very much more sensitive than those taken simultaneously by the wet and dry bulb thermometer of the usual type. Now, obviously, the most ideal atmosphere for good health and comfort is out of doors, which indicates that in rooms we should endeavor to imitate the outside conditions with regard to cooling power as closely as possible. We should aim not only at air which can cool the surface of the body at a rate that is proportional to the rate of heat production, but we should imitate the outside variability in cooling power. Monotony of cooling conditions in a

room is unnatural and should be avoided if the inhabitants are to remain alert. On this account Hill is inclined to recommend for ordinary class rooms that they should be heated by low pressure steam pipes with open windows instead of being heated and ventilated by the plenum system. For larger meeting places, however, a plenum system with frequent inlets and outlets must be used.

It is, of course, admitted that observations on ventilation made in the British Isles with their relatively constant temperature throughout the year, are not directly applicable to the American continent with its extremes of heat and cold. Before the results can be made applicable, there must be independent observations on this continent, and the practical question in ventilation at present is to have a sufficient number of such observations made so that definite conclusions may be drawn. Much useful work in this direction has already been done by the New York Commission on Ventilation and in the public schools of Chicago, but much more work is required. In a general way the problem is to compare measurements made by physical instruments (Kata thermometer) of the cooling effect of the air in various parts of the hall or room with the well-being and comfort of the individuals that occupy it. It is with regard to the quantitative evaluation of comfort that the greatest difficulties arise, for, to make the comparisons of any value it is necessary to adopt certain more or less arbitrary standards. The New York Commission used various psychologic and physiologic tests and in school rooms the responsiveness and quickness of the pupils in answering questions have been useful, but for practical purposes it is probable that much useful information could be collected by merely asking the individuals their opinion of the ventilatory conditions and recording their impressions in general terms. By collecting large numbers of observations, errors of judgment would be ruled out.

With regard to the measurement of the cooling power, the ideal instrument to use is of course the Kata thermometer but there is one objection to it, namely, that it demands some time and care to obtain an adequate number of readings. If readings were to be taken at various parts of a hall, for example, it would necessitate observers at all these places. To make the instrument more practical Hill has therefore more recently devised Kata thermometers that are kept constantly heated by an electric heating device, the simplest form of these being the so-called "comfimeter." This consists of a metal tube which widens below to a cylindrical chamber near the upper end of which (an inch or so from the tube), is a metal partition carrying on its lower face a socket for an electric (carbon) lamp. Just above the partition the walls of the cylinder are provided with openings. The lamp heats the air in the cylinder above the partition and this causes cool air to enter by the openings and ascend the tube, and the temperature of this ascending column of air is measured by the thermometer which hangs in its center. It is clear that the temperature of this air, the heating power of the lamp remaining constant, will depend on the cooling influence of the atmosphere. We have made numerous comparisons between the readings by the comfimeter and those by the Kata thermometer and have found that they correspond with



entirely satisfactory closeness. By using several confimeters throughout a class room we propose to compare the readings with the collective opinion of students sitting near, as to the ventilatory conditions. The instrument is so easy to use that it should be possible to have large numbers of observations recorded and thus perhaps to have at last a reliable method by which the efficiency of a system of ventilation could be tested.

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—J. J. R. M.

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*Experimental Influenza Bacillus Infection in Man*

WITH the lapse of time since the 1918 influenza epidemic we find progressively less literature appearing on the subject. This is but natural. The storm having passed, we are inclined to forget it. Interest will lag more and more as time goes on until, with the advent of the next pandemic prevalence we will once again be taken unawares. If the interval be sufficiently long we will first pass through a period of conjecture as to what the strange new disease is, then one in which we suspect that it may be our old friend influenza, and finally after it has run a great part of its course we will decide that it is influenza, and will hasten to combat the pestilence with latest and most approved methods. The disease will, as in the past, pursue its course, little influenced by our frantic efforts.

During and after the next pandemic the literature will again be flooded with clinical description, with epidemiologic data, with immunologic observations, and with disquisitions pro and con, as to whether the disease is identical with that which swept the world in 1918.

In the case of influenza, we appear indisposed to profit by the experiences of past epidemics. Innumerable observations have been made since 1918, observations and theories which are believed by their sponsors to be entirely new and unprecedented. It was stated that influenza affected different age groups in 1918 from those chiefly stricken in 1889 and 1890. It has been stated that the students of the 1889 epidemic believed in an origin from an endemic focus in Turkestan. Now it is believed that the disease is widely distributed in endemic form during the interepidemic period. If one will but take the time to study the records following the 1889 epidemic, one will discover that the clinical and epidemiologic characteristics were the same, that the age incidence was the same, in short that the disease was true to type in its essential manifestations; also that the possible or even probable universal distribution of a virus of low virulence during the interepidemic period was fully discussed after the earlier pandemic. Even on the subject of bacteriologic etiology we are little farther advanced today than we were 25 or 30 years ago.

At that time it was quite generally conceded that the Pfeiffer bacillus was the cause of the disease. Yet there were many dissenters. Today we admit that we know nothing definitely of the causative agent but the discussion remains now as formerly, whether the influenza bacillus is or is not the cause of epidemic influenza.

If we are to profit by the disastrous experiences of the last epidemic; if we are to be better prepared to combat the ravages of influenza, it will be important that the disease be not completely forgotten during the next few years. Influenza and associated or similar conditions must be studied from all points of view, from that of the clinician, the bacteriologist, the immunologist and particularly the epidemiologist. Until the time when a specific vaccine, immune serum or other similar substance has been developed, greatest hope for the moderation of epidemic prevalences rests with the work of the epidemiologist. There are diseases, which like smallpox, have been brought under control, even though we know little regarding the causative germ. Incidentally we may expect considerable aid from the development of a vaccine directed against the secondary invaders.

It will be of considerable help if the rôle of the influenza bacillus can be fairly definitely established. There is some evidence indicating that this microorganism is not the primary cause of the disease. The evidence is, however, chiefly of a negative character. It has been claimed by many that the bacillus is of no pathogenic significance whatever. The work of Blake and Cecil, of Cecil and Steffen, and of Parker, has done much to establish the pathogenicity of the influenza bacillus.

Blake and Cecil inoculated the nose and throat of monkeys with a virulent culture of influenza bacillus and produced an acute upper respiratory tract infection which was quite similar to influenza as it occurs in man. The experimental infection was characterized by rapidity of onset, marked prostration, fever, leucopenia and a certain degree of contagiousness. Some of the cases developed secondary involvement of the lower respiratory tract. This work indicated that in virulent form the influenza bacillus is capable of producing an acute inflammation of the upper respiratory tract in monkeys. Parker demonstrated that a soluble toxin is formed in culture by the influenza bacillus, which is fatal on inoculation into rabbits.

Cecil and Steffen have continued the experiments of Blake and Cecil by attempting to transmit infection to man. They were able to produce an acute infection of the upper respiratory tract by the application of influenza bacillus cultures to the mucous membrane of the nose and throat. The recently isolated germ had been grown on chocolate blood broth medium and was used when the growth was six hours old. They likewise produced symptoms after the application to the upper respiratory mucosa of small amounts of peritoneal exudate from a monkey, dying from influenza bacillus peritonitis. Cultures passed through Berkefeld filters produced no symptoms whatever when applied in the same manner. The infection produced was characterized by the symptoms of acute coryza, with considerable prostration, and occasionally with headaches or other general pains. There was an associated leucopenia. As stated, these symptoms were not produced by filtrates and they were not

produced when the hemolytic streptococcus was substituted for the influenza bacillus. In the latter case, acute follicular tonsillitis, with leucocytosis, resulted. Pneumococcus cultures applied in the same way produced no symptoms and no elevation of temperature.

By their work Cecil and Steffen have shown on a small number of cases that the influenza bacillus in virulent form can produce an infection of the upper respiratory tract simulating in its clinical characteristics either acute coryza or a very mild influenza. None of the experimental cases developed an increased temperature, but they point out that the fatal case of influenza pneumonia, from which the virulent culture was isolated, also ran a very low temperature during the period under observation. They have also shown that the influenza bacillus when inoculated onto the mucous membrane usually disappears within a short time but that it may remain present for several days or even weeks after the inoculation.

The work of these various observers has shown that a virulent influenza bacillus can cause an infection of the upper respiratory tract in man. The organism may at times be quite highly invasive and may, under experimental conditions, at least, be a primary invader. It has not shown that the influenza bacillus is the cause of epidemic influenza. The work has carried our knowledge a step forward by demonstrating that at all events the influenza bacillus is not an organism to be lightly disregarded and that, no matter what its importance as a secondary invader in influenza, it must be regarded as a potential cause of primary acute infection of the upper respiratory tract.

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—W. T. V.

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### *Silver Arsphenamine*

FOR some time accounts have appeared in medical literature of a new arsenic preparation used in the treatment of syphilis. This has been called "silver salvarsan, or more precisely the sodium salt of silver-diaminodihydroxy arsenobenzene, and it contains approximately 22.5 per cent of arsenic and 14 per cent of silver. It is presumed that the silver for which spirochetes have an especial affinity serves as an anchor for the arsenic, and that therefore the drug, despite its lower arsenic content than arsphenamine, is more active therapeutically.

Animal experimentation seems to show that silver salvarsan is twice as effective as the old salvarsan (606) and three times as effective as neosalvarsan (914). Kolle states that silver salvarsan is old salvarsan in active form plus silver, and that 0.25 silver salvarsan is the equivalent of 0.4 of old salvarsan.

Dreyfus in discussing his experience with this drug in treatment of syphilis of the nervous system says that silver salvarsan is three times as efficacious as

the old salvarsan, that it acts more quickly, its toxic dose is higher, and it has the advantage of a combined silver and arsenic effect. He says that in nervous syphilis the indications are that the new preparation promises to be more valuable than the older ones, even when the latter are used in conjunction with mercury. It is Dreyfus' experience that, especially in early cases, both subjective and objective symptoms show marked signs of retrogression within two weeks. More care is necessary in tabes than in other conditions, but in all, small doses with carefully graduated increases are used.

Boas and Kissmeyer, after treating 62 cases representing all stages of syphilis, and using mercury with it, found that silver salvarsan was just as effective as old salvarsan, and in addition it was more soluble and easier to handle. They prefer it for these reasons only. Körsbjerg on the other hand is very enthusiastic over the new drug and has been so impressed by its effects that neither he, nor his chief Jersild, use mercury either as an accompaniment nor as a follow up treatment. There were 52 cases in the series reported, of which 19 were secondary. In every case all symptoms had vanished within two weeks of the first injection.

The use of this silver-arsenic preparation seems from the reports to be attended by more danger than the older preparation. This danger is reflected in the dosages used, i. e., from 0.02 to a maximum of 0.25, in dilute solution. Anaphylactoid symptoms,—redness and swelling of the face and buccal mucous membrane,—pyrexia; cutaneous eruptions which are usually transient, and occasionally severe dermatitis; syncope, collapse, vomiting, vertigo and headache; and icterus are all listed as secondary effects.

Neurorecurrences seem from reports to be fewer after silver salvarsan than after the older drugs, but the cases are too few to be decisive.

A large series of cases treated with silver salvarsan is that of Behring, who reports upon his experience in giving 5,200 injections in 259 cases. In this series icterus occurred 9 times, and angioneurotic symptoms 6 times. There was one death. In his experience no venous thrombosis occurred. Eruptions and icterus were not more commonly observed after silver salvarsan than after the older preparations. Silver salvarsan is well adapted to the abortive treatment of syphilis. Behring says that a definite verdict cannot as yet be given with reference to tabes and central syphilis, although tabes is apparently favorably influenced.

Wiener says that the curative effects in primary lesions, secondary and tertiary symptoms are very favorable and apparently not inferior to the results of a combined course of neosalvarsan and mercury in the customary dose.

The most recent report on this silver-arsphenamine is based upon the experience of Major Watson of the Medical Corps, U. S. A., and has to do with the treatment of 800 patients, and more than 6000 injections. The method of treatment recommended by the Board of Medical Officers, and the method used by Watson was as follows:

An interval of seven days between each dose in each course of treatment. Treatment to consist of four courses of silver salvarsan and gray oil.

In the first course of treatment the first dose to be fifteen hundredths

(0.15) gm. of the drug. The second dose to the two tenths (0.2) gm., and each of the remaining five doses of the course to be three tenths (0.3) gm. of the drug.

At the end of the first course of treatment a Wassermann blood test is made and then thirty days' rest.

In the second course of treatment three-tenths (0.3) gm. of the drug is given at each of seven injections, at seven day intervals, and is followed by two and one-half months' rest.

The third and fourth courses are the same as the second, with ninety days' interval between the two. Gray oil is used in conjunction with and at the same time as each injection of silver salvarsan, using eight hundredths (0.08) gm., by intramuscular injection.

A blood Wassermann is recommended after each course and a spinal fluid Wassermann after the second.

Such is the army intensive treatment. As against it Hoffmann says that one complete intensive course of treatment with mercury and silver salvarsan in primary syphilis will give a complete cure. Hoffmann, Neisser and Scholz believe that if treatment can be commenced in the prepositive Wassermann stage the disease can be cured in from 80-100 per cent of the cases. Nevertheless it is to be borne in mind that all persons who have had syphilis, no matter how rapidly the clinical signs disappear, nor how soon the Wassermann reaction becomes negative, should be watched both for clinical and serologic recurrences.

It seems from a review of the literature, that in silver-arsphenamine we have a more potent antispirochetide than any heretofore in use, and one which should be used with the greatest care. It seems to represent a real therapeutic advance.

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—P. G. W.

### *International Organization and Public Health*

BUCHANAN<sup>1</sup> discusses the League of Nations and Public Health. It is evident that, while all medical science, both preventive and curative, is international and free for any one who chooses to use it, still a League of Nations might do many things in order to improve the living conditions of the populations of its constituent members. If all the nations of the earth, or even if the great nations of the earth, should combine in efforts to stamp out infectious diseases, great good could be accomplished. Since 1903 there has been an agreement among the great nations to notify all others when certain infectious diseases, notably the plague and Asiatic cholera, occur in any one country. Too frequently these notifications pass through diplomatic channels

<sup>1</sup>Lancet, London, 1921, cc, 415.

and, like all procedures depending upon red tape, are slow in their dissemination. Even the war left untouched, largely at least, one international prerogative, and that is, the right of any nation to protect its people from the importation of infection. For centuries there has been more or less conflict in regard to quarantine laws between the protection of health on one side and the interference with commerce on the other. An example of how improvement might be brought about is at hand in the distribution of anthrax quite broadly over the world from Japan in recent years through shaving brushes. Several countries have found it necessary to take action in this matter. If Japan would guarantee the thorough sterilization of its exported articles, there would be no danger of carrying infection to other countries, and at the same time there would be no necessity for other countries to exclude certain importations from Japan. Recently, the British Government has been obliged to prohibit the importation of shaving brushes from Japan. The United States has, we believe, not been quite so radical in dealing with the Japanese, but has demanded that all shaving brushes brought from Japan into this country should be sterilized. That this has not absolutely stopped the importation of anthrax-laden brushes is shown by the fact that now and then infection through this agency is reported. Each government must rely upon its own consular service, largely at least, in protecting itself from infection from abroad. When we realize that consuls, as a rule at least, have no knowledge of health matters and often no interest in them, it is easy to see the inadequacy of this provision. The International Red Cross has taken up its residence in Geneva, has built certain laboratories, is collecting a library, and is publishing in several languages, a fairly creditable journal. This work, however, is voluntary, is dependent upon uncertain support, and up to the present time at least, has no common or universal sanction. Buchanan states that the League of Red Cross Societies has already found itself hampered by the absence of a health section at the League of Nations. Since 1907 there has been a semi-official International Health Publication issued in Paris. This was established by the convention held at Rome. This central office has been of great value, and it would be unfortunate if it should not be chosen as the nucleus of a greater International Health organization.

Much might be accomplished by the internationalization of vital statistics. The basis on which these are collected in the different countries is widely variable and one must be careful in drawing conclusions from statistical data secured in this way; in fact, there is no country in the world where vital statistics, even concerning births and deaths, are rigidly correct. The best we can say is that in some countries they are approximately correct. Even in the same country, the nomenclature of disease changes from time to time and in more than one instance this has deceived medical writers who have concluded that, because a given name no longer appears in the mortality statistics, the disease which it represents has ceased.

—V. C. F.

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## ORIGINAL ARTICLES

### THE PATHOLOGY OF INFLUENZA AS SEEN IN THOSE WITH CHRONIC MENTAL DISEASE\*

BY NOLAN D. C. LEWIS, M.D., WASHINGTON, D. C.

A LARGE number of excellent reports with accurate descriptions of the lesions in various organs taken at autopsy on individuals dead from influenza have been written by authorities of many countries and particularly of Military General Hospitals, but as these accounts deal largely with material which was supposedly approximately healthy before the onset of the disease, it was thought expedient to study the condition when superimposed on depleted tissues which were partially disorganized by other diseases.

The following investigation was carried out on autopsy material obtained at the Government Hospital for the Insane, Washington, D. C.

This group of 42 cases is particularly interesting because (1) in most instances the acute infection occurred in tissues already considerably impaired by the chronic processes usually existant in psychopathic and neurologic patients, (2) special attention has been given to the histopathologic changes in the central nervous system; and (3) the ages of the individuals studied range from 7½ months (fetus) to 78 years, with many cases past middle life, and 5 cases over 70 years of age.

Table I is given to indicate the age, the sex, the original lesions, and the psychosis.

#### EXTERNAL APPEARANCES

At the examination of a body dead from influenza, one is impressed with the general picture of asphyxia; the frothy exudate from the mouth and often from the external nares, the lividity about the face, neck, and shoulders and the cyanosis of the nails; all point toward an acute asphyxiative condition. Often rigor mortis is rapid in onset and of prolonged duration.

\*From the Government Hospital for the Insane, Washington, D. C.

## MUCOUS MEMBRANES

The mucous membranes present two types of change which in the mouth may be present even as far as the mucocutaneous junction of the lips, which are then a striking bright red in color. In type 1 the trachea and bronchioles contain a frothy semifluid, blood tinged exudate, and the lining membranes are characterized by networks of dilated, plainly visible, bright red capillaries.

Microscopically we see the lining cells in stages of albuminous degeneration with some areas of death and desquamation. The capillaries are seen to be stretched almost to the point of rupture, being densely packed with red blood corpuscles.

The mucous membranes of the esophagus, stomach and intestines, are usually of this type; although some have spoken of the submucous hemorrhages of petechial nature, as being frequent in influenza, it should not be connected in any special way, as such reactions are noted in many diseases not particularly related. The membranes of the bladder and genital organs are also usually of the Type 1.

In Type 2 there is an intense congestion, rupturing the vessel walls, with an extensive pouring out of corpuscles producing a general diffuse, glairy, beefy red color to the structures. This variety of change is probably due to defective oxygenation of the blood, producing an acidosis which affects the stability of the vessel walls. The microscope discloses a very striking and characteristic picture. The blood vessel walls of the submucosa are under tension, the muscle fibers appear dead and unstained, the nuclei of the cells very pale, and the endothelial lining cells dislodged. Large numbers of vessels with these deficient walls have ruptured and the red blood cells have poured out into the surrounding substances, where focal necroses are produced from pressure and lack of nutrition. Endothelial leucocytes gather in the vicinities of these hemorrhages and ingest the masses of blood pigment from the disintegrated red cells. In the trachea and bronchi the epithelium may be indistinct and in places completely degenerated, there remaining only a faint outline of former epithelial structure. Submucous tissues are edematous, congested and contain many lymphocytes. The glandular areas contain numerous lymphocytes, much pigment deposit, dilated vessels, and a few acute inflammatory cells.

## SEROUS SURFACES

*Pleurae.* The parietal pleura presents a diffusely reddened surface over which the dilated blood-filled capillaries stand out prominently. The visceral pleura is usually darkened and frequently exhibits petechial or confluent hemorrhages.

Some have claimed that the pleura is free from exudate and is usually without fibrin deposits; but in this group of cases a thin layer of fibrinous exudate, especially between the lobes of the lungs, could practically always be demonstrated when selective staining methods were employed.

In cases of chronic pleuritis of ancient origin, new formation fibrin is pres-



ent in large amounts (see Fig. 1) and the bands of adhesion are strikingly hemorrhagic.

Due to rupture of the surface alveoli of the lungs, many air blebs occur beneath the pleurae.

The pleural fluids vary greatly in amount and character; in some instances as many as 500 c.c. of clear yellowish fluid are present, but usually the pleural cavity contains only a few cubic centimeters of blood-tinged slightly turbid fluid, which in cases associated with jaundice presents the additional bile tint.

*Pericardium*.—A moderate serous effusion is almost always present, with the fluid sometimes clear, but more often blood-tinged. Both layers of the pericardium are most intensively congested and occasionally a case is found presenting distinct petechial hemorrhagic spots usually distributed along the left side of the sac.

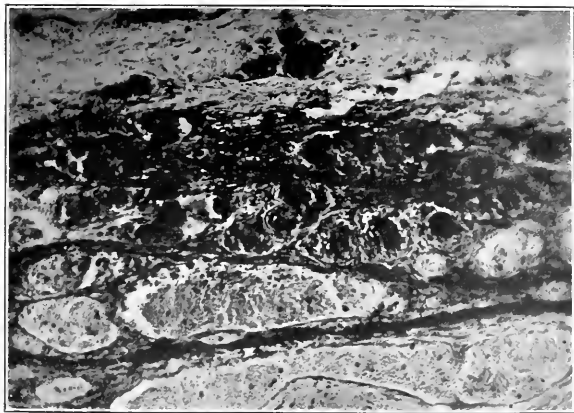


Fig. 1.—Hemorrhages and strands of fibrin in sections of chronic pleuritis.

*Peritoneum*.—There is an intense congestion of the entire peritoneum with all minute vessels standing out prominently. The vessels of the intestinal surfaces are frequently so engorged as to produce a contrast not unlike that effected by an exaggerated over-colored painting.

#### LUNGS

Because of the prevalence of chronic pulmonary conditions, particularly the secondary indurative pneumonias, in this class of individuals, the lung findings are not easily arranged into the fairly distinct types usually described in connection with this disease; however, the acute inflammatory edema or hemorrhagic pneumonitis is easily demonstrated in those dying shortly after the onset of the disease. In these cases, regardless of the type or extent of chronic condition present, about 90 per cent of the lung tissue is involved

in a process characterized by a rounded dark red, firm, edematous surface without lobular distribution.

An enormous accumulation of bloody fluid is present in all portions, and on section streams of this fluid often to the amount of 100 to 200 c.c. pour spon-

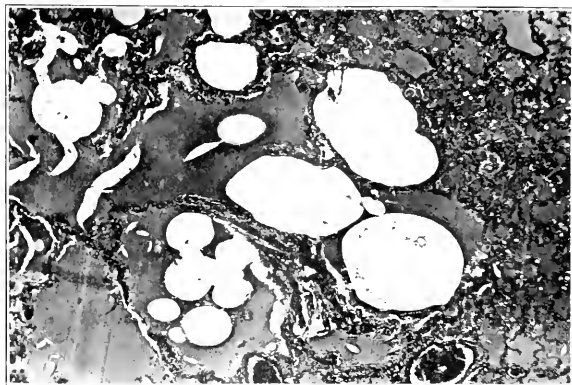


Fig. 2. Acute inflammatory pulmonary edema with alveoli widely distended with acellular fluid.

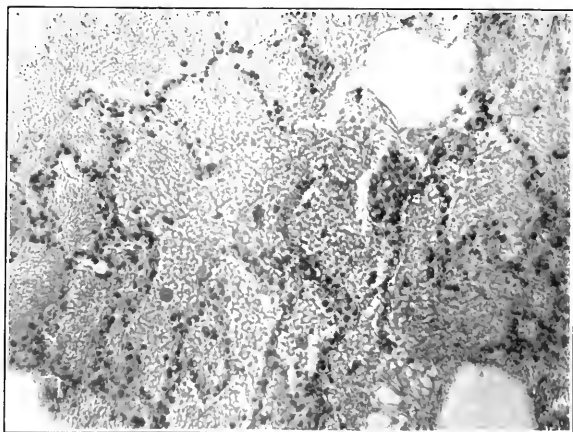


Fig. 3. Congestion of a blood vessel with free red blood corpuscles.

taneously from the incision. The cut surfaces present a somewhat irregular appearance with patches of slightly emphysematous alveoli alternating with larger areas of edematous hemorrhagic tissue.

*Microscopical.* In cases free from previously existing pulmonary lesions the alveoli are moderately dilated and the walls are outlined by deeply in-

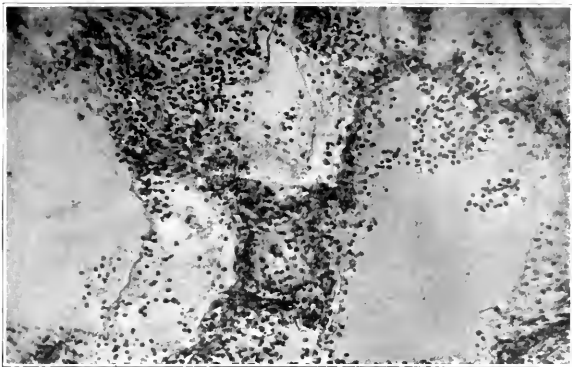


Fig. 4—Congested alveolar walls surrounding areas of fluid containing a few polymorphs.



Fig. 5—(Low power). Alveoli filled with mixed cellular exudate. The darker areas are masses of fibrin stained with phosphotungstic acid hematoxylin.



Fig. 6. Infiltrated interstitial tissue with an aneurysm containing masses of pus cells.



Fig. 7. Alveoli outlined by fibrous strands. Lumen largely composed of red blood globules, or blood content.

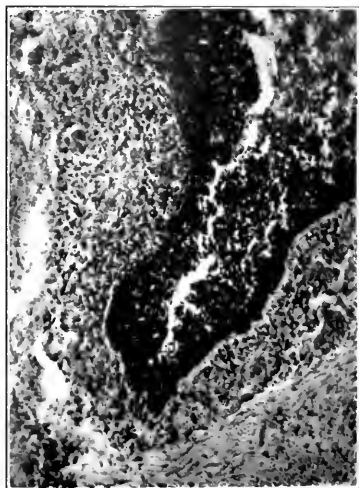


Fig. 8. Section of portion of bronchus packed with cellular exudate. Walls are infiltrated with mononuclears and red blood cells.

jected arterioles. The air spaces contain either a homogenous clear acellular exudate (Fig. 2) or are packed by free red blood elements, (Fig. 3) and the lining cells are usually greatly enlarged and distorted by large single or multiple aqueous globules in the protoplasm.

The bronchioles generally contain a granular exudate in which there are a few red blood globules and detached epithelial elements.

In some cases, groups of air sacs are dilated to the point of fracture of the thin walls, while other groups are normal in size, but contain an homogeneous, gelatinous-appearing exudate with a few polymorphonuclear leucocytes scattered through the substance. (Fig. 4). In this setting the bronchi are always dilated, often filled with free blood and the walls are surrounded by mononuclear leucocytes.

In other cases the walls of the alveoli and the whole lung substance appear to be a mass of blood pigment, granular exudate, congested vessels, and newly formed fibrin.

Another type of lung condition frequently found is manifest by a few elevated patchy consolidations beneath the cyanotic edematous surface. Usually also there is a thin surface deposit of fibrin, particularly between the lobes. The substance of the lung is filled with dark purplish blood, and beginning elevated consolidations, firm and resistant to touch, are distributed in the central portions about the main bronchi near the hilum of the organ, from which point they diminish in size and number toward the apex and base. The bronchi contain a thin blood-tinged exudate, which in some cases is slightly purulent; the lumina is in many instances distinctly narrowed by the swelling of the mucous membranes. Blood capillaries of all tissues show most intense congestion, and the larger vessels are often filled with clots.

*Microscopical.*—Through these consolidated areas skeins of fibrin are strewn and lymphocytes are everywhere abundant. (Fig. 5.) In small localities there are groups of alveoli entirely filled with polymorphonuclear leucocytes; (Fig. 6) these alternate with others, which contain a granular exudate filled with red cells. (Fig. 7.) Catarrhal cells are numerous in all sections.

In some areas the bronchi are packed with pus cells, red blood globules and detached epithelial cells (Fig. 8). In lungs, the seat of bronchiectasis, the larger vessels are engorged with blood, the alveoli are thickened and heavily outlined by red blood corpuscles, and around the larger bronchi dense consolidation with acute inflammatory cells is present. In the lower lobes large areas of alveoli are filled with polymorphs.

When tuberculosis in any form is present, and where fibrous tissues are greatly increased, the tissues are especially rich in deeply congested vessels surrounded by small mononuclear cells. Highly pigmented catarrhal cells are very numerous.

#### THE HEART AND GREAT VESSELS

The majority of hearts in this series is the seat of a chronic interstitial myocarditis with tortuous, congested, atheromatous coronaries and nodular incompetent valve cusps.

In some cases all cavities are filled with large, red clots which sometimes

extend upward into the carotid branches and are whipped into the cordæ tendinæ.

Usually the right heart and whole pulmonary venous system is dilated and choked with fluid blood; and the auricular appendage often contains an enormous clot which strings along through the dilated ring of the tricuspid valve into the right ventricle where occasionally the cordæ tendinæ are very fragile and have ruptured under the increased strain.

In a few cases the muscle substance shows only a moderate cloudy swelling of the fibers, but in the greater number the tissue is very pale, softened and flabby.

When the valve cusps are free from atheromatous changes, they are apt to exhibit multiple minute hemorrhages.

TABLE I  
INDICATING PRINCIPAL LESIONS AND PSYCHOSIS

NO.	AGE	SEX	ORIGINAL LESION	PSYCHOSIS
1	58	M	General Arteriosclerosis	Catatonic Dementia Precox
2	56	F	General Arteriosclerosis	Arteriosclerotic Dementia
3	40	M	Chronic Ulcerative Pulmonary TB	Paranoid Dementia Precox
4	50	M	General Syphilis	Cerebral Syphilis
5	72	M	Cerebral Arteriosclerosis	Manic Depressive Insanity
6	38	F	Massive Ascites	Dementia Precox
7	74	M	Pulmonary Abscess	Senile Deterioration
8	29	M	General Syphilis	Dementia Precox
9	57	M	General Arteriosclerosis	Dementia Precox
10	43	M	Chronic Ulcerative Pulmonary TB	Dementia Precox
11	45	M	Chronic Endo and Myocarditis	Undiagnosed
12	60	M	Osteomalacia	Senile Deterioration
13	47	M	Ancient Hemiplegia	Organic Brain Disease
14	50	M	Thyroid Hyperplasia	Dementia Precox
15	54	M	Chronic Ulcerative Pulmonary TB	Epileptic Psychosis
16	Agcd	M	Fibrous Pleurisy	Epileptic Psychosis
17	59	F	Cerebral Arteriosclerosis	Arteriosclerotic Dementia
18	50	F	None	Dementia Precox
19	35	M	Early Miliary Pulmonary TB	Dementia Precox
20	21	M	Miliary TB (Pulmonary)	Dementia Precox
21	50	M	Diffuse Productive Nephritis	Arteriosclerotic Dementia
22	49	M	Chronic Myocarditis	Dementia Precox
23	43	M	Gangrene of Lung	Undiagnosed
24	64	M	General Arteriosclerosis	Undiagnosed
25	24	M	Chronic Pleuritis	Dementia Precox
26	49	M	Productive Nephritis	Dementia Precox
27	15	M	General Paresis	General Paresis
28	35	M	General Syphilis	Depression
29	38	M	Chronic Parenchymatous Nephritis	Dementia Precox
30	53	M	Generalized Miliary TB	Dementia Precox
31	44	M	Chronic Myocarditis	Dementia Precox
32	32	M	Bilateral Pyothorax	Dementia Precox
33	29	F	Chronic Ulcerative Pulmonary TB	Dementia Precox
34	78	M	Cerebral Arteriosclerosis	Senile Deterioration
35	71	M	Cerebral Arteriosclerosis	Senile Deterioration
36	70	M	Cerebral Arteriosclerosis	Arteriosclerotic Dementia
37	68	M	General Arteriosclerosis	Senile Deterioration
38	45	M	Chronic Ulcerative Pulmonary TB	Dementia Precox
39	48	M	Productive Nephritis	Dementia Precox
40	21	F	Miliary Pulmonary TB	Imbecility
41	7½ Mo.	M	None	
42	38	M	Internal Hydrocephalus	Imbecility

*Microscopic.*—In the most acute cases (i. e., short typical course with early termination) the capillaries are round, dilated and filled with red blood glob-

ules. In many zones the thin-walled structures have ruptured, allowing blood to diffuse into and between the muscle fibers which may be pigmented and widely separated.

In a few cases the heart muscle fibers are separated by long strings of corpuscles in rouleaux formation, the capillaries being intensely injected, the coronary arterioles packed with cells, and an occasional red thrombus present in the larger coronary branches.

Where the muscle cells have undergone extreme compensatory hypertrophy in chronic myositis there are separation, fragmentation, and albuminous degeneration of the fibers. In this extreme type the vascular changes are much less in evidence.

In other specimens focal separations of muscle occur, the spaces being filled with red blood corpuscles and hemoglobin pigments; in these there is much congestion and acute change in the cardiac capillaries.

Occasionally only clonding of the muscle fibers is noted. Acute clondy changes in the cells, indistinct nuclei, increase in sheath elements and a moderate thickening of the vessel walls characterize the picture. In some cases of influenza in which there has been a previous myocarditis the only change present microscopically is that of chronicity, the acute process having apparently left no traces.

#### LIVER

In this series twenty-seven cases presented acute congestion with beginning fatty changes in the liver substance. In general, the organ was slightly enlarged, softened and edematous with yellowish patches on the surfaces. Cut surfaces were pale, yellowish and patchy yellow, with blood clots in the larger veins.

A few cases presented a deeply hemorrhagic substance containing much residual blood and in general cyanotic in appearance. Four cases showed a marked acute passive congestion and in three cases of portal cirrhosis, gross acute changes could not be demonstrated.

*Microscopic.*—The portal veins are notably dilated, and sometimes are distended almost to the point of rupture, the intralobular veins are packed with corpuscles, and the surrounding hepatic cells which are separated by clumps or masses of red blood corpuscles are undergoing acute fatty alteration.

The bile ducts are dilated, the lining cells in states of degeneration, and there is diffuse granular pigment scattered through the tissues, especially in cases where the chronic cellular elements are notably increased in the portal canals. The bile pigments are markedly increased in the organs, presenting a thickened capsule, retrograde hepatic cells, proliferating bile capillaries and new formation connective tissue.

Occasionally the hepatic cells are widely separated and distorted, showing extreme fatty alteration, many being mere rims of protoplasm, the center of which is filled with large globules. The shrunken cells are separated by a finely granular deposit which contains many red blood cells.

Central lobular acute cellular changes are frequent and often the cells are swollen edematous and indistinct in nuclear outlines.

Wherever fibrous tissue has previously formed as in portal cirrhosis, the newly formed blood capillaries stand out prominently from congestion. The thickened fibrous capsules are well studded with congested capillaries and the tissues contain many free blood cells. In these situations where islands of liver tissue have long been exposed to pressure from the strands of thickened connective tissue, the cells show localized areas of acute destruction; the spaces left from the complete necroses are occupied by clumps of red blood corpuscles from the surrounding ruptured vessels.

#### SPLEEN

The most frequent changes noted in these spleens are moderate enlargement, cyanotic surfaces often with dark mottlings, and rather firm on palpation; and a bright, softened, easily fractured substance.

Sections disclose a soft, swollen pasty pulp loaded with semifluid blood. Twenty-eight cases are of this variety.

In four instances hemorrhagic perisplenitis with pigmentation of splenic substances occurred. In only one case is the spleen pale in color, and in this the softening of the pulp is well marked.

In seven cases the spleen was originally of the chronic interstitial type, but presents acute congestion, a creamy pink pulp and a softening of the lymphoid structures.

*Microscopic.*—In the acutely congested types there is an excessive amount of blood pigment distributed between the cells of the pulp, the sinuses engorged with red blood cells or granular debris, the endothelial cells in stages of proliferation, and the arterioles exceptionally large and filled with blood. Instead of the diffuse picture the splenic pulp may be dotted by irregular patches of dense hemorrhage, with a striking congestion of the arterioles of the malpighian bodies.

Hyaline degeneration of the vessels is relatively frequent and the lymph nodules may be surrounded by hemorrhagic zones.

When there is a chronic splenitis with an increase in trabeculae, the blood vessels of this tissue stand out very prominently from congestion, and beneath the thickened capsules, zones, filled with minute hemorrhages are frequent. Many endothelial phagocytes bearing blood pigments are scattered in these regions.

The larger vessels in general present a pale, homogeneous-appearing media and an occasional hyaline degeneration or thrombotic formation.

#### KIDNEYS

In nearly all the cases, regardless of the original type of lesion present in the kidneys, an acute change could easily be demonstrated. (Table II.) The organs are slightly tense from swelling, and general congestion; the stellate veins deeply injected; the cut surfaces edematous, granular, everted, often fatty, and deeply hemorrhagic; the pyramids and papillae enlarged and congested, and the larger vessels often filled with dark red blood clots.

*Microscopic.* Microscopically, they may be arranged into two groups, viz.:—



Group 1, where the changes are acute only, and Group 2 with two subdivisions, where acute processes are superimposed upon chronic kidney disease.

In Group 1 the interstitial tissues, including the capsule, contain minute hemorrhages, where the small capillaries have ruptured and thrown their

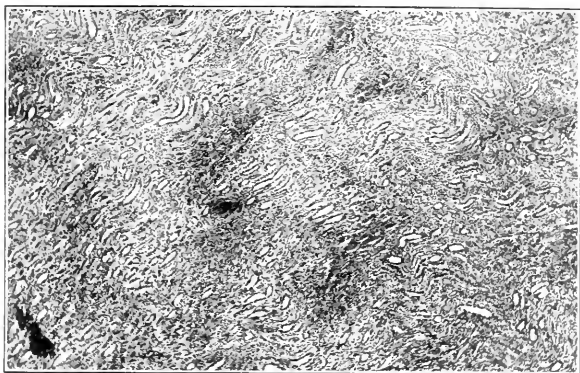


Fig. 9. (Low power.) Dark areas indicate intratubular and intertubular hemorrhages.

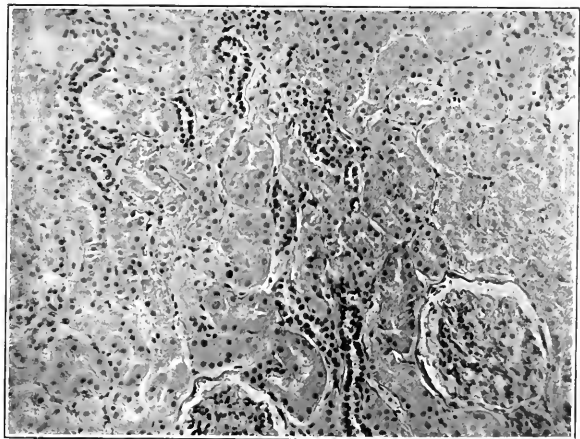


Fig. 10.—Simple cloudy swelling of the parenchyma.

contents into the surrounding tissues. Hemorrhage is usually most profuse immediately beneath the capsule, where masses of free blood corpuscles are distributed over wide areas. Intertubular hemorrhages are present in all fields, but are more abundant through the collecting tubule regions where the thin-walled, congested capillaries have partially disintegrated. (Fig. 9.)

Some glomeruli are very hemorrhagic, the spaces filled with finely granu-

TABLE II  
ACUTE CHANGES IN THE KIDNEYS

*Group I. Without Chronic Kidney Lesions.*

CASE NO.	EDEMA AND CONGESTION	ACUTE PARENCHYMATOUS CELL CHANGES	HEMORRHAGES
3	Slight	Slight	None
8	Intense	Advanced	Numerous intratubular hemorrhages and hemorrhagic tufts
10	Marked	Moderate	Diffusely hemorrhagic substance
11	Intense	Moderate	Hemorrhages into glomeruli and into interstitial tissues
14	Marked	Advanced	Few hemorrhages through collecting tubules
16	Moderate	Moderate	Minute hemorrhages through interstitial tissues
17	Marked	Moderate	Numerous intra and intertubular hemorrhages
18	Slight	Slight	None
19	Slight	Advanced	Intratubular hemorrhages
20	Marked	Marked	Intratubular hemorrhages and hemorrhagic glomeruli
21	Marked	Advanced	Intratubular hemorrhages and hemorrhagic glomeruli
22	Marked	Slight	None
25	Marked	Advanced	Very numerous intertubular hemorrhages
27	Marked	Slight	Few hemorrhagic glomeruli
29	Slight	Slight	Few ruptured capillaries
30	Moderate	Slight	None
31	Marked	Moderate	Hemorrhages into tufts
32	Marked	Moderate	Hemorrhagic glomerulitis and intertubular hemorrhages
40	Marked	Marked	Hemorrhagic glomerulitis
41	Marked	Marked	Patchy interstitial hemorrhages
42	Marked	Marked	Intertubular hemorrhages

*Group II (a) With Chronic Kidney Lesions (Diffuse Productive Nephritis).*

1	Marked	Advanced	Hemorrhagic interstitial tissues
4	Marked	Advanced	Enormous number of small hemorrhages in all structures
6	Marked	Advanced	Very numerous intertubular hemorrhages
9	None	Moderate	None
12	Moderate	Advanced	Numerous interstitial hemorrhages
13	Marked	Marked	Diffuse hemorrhages between tubules and into glomeruli
15	Marked	Slight	Hemorrhagic glomeruli
23	Marked	Advanced	Hemorrhages into tufts and into interstitial tissues
24	Moderate	Moderate	Few hemorrhages into glomeruli
26	Marked	Advanced	Numerous subcapsular hemorrhages
28	Marked	Advanced	Diffusely hemorrhagic substance
33	Moderate	Moderate	Glomeruli moderately hemorrhagic
34	Marked	Marked	Few small intertubular hemorrhages
35	Marked	Marked	All portions deeply hemorrhagic
36	Marked	Marked	All portions deeply hemorrhagic
38	Marked	Marked	Hemorrhagic glomeruli
39	Marked	Marked	Small inter and intratubular hemorrhages

*Group II (b) With Chronic Kidney Lesions (Patchy Productive Nephritis).*

2	Moderate	Early stages	Diffuse hemorrhagic glomerulitis
5	Marked	Moderate	All glomeruli hemorrhagic—patchy intratubular hemorrhages
7	Slight	Slight	None
37	Marked	Marked	Numerous intertubular hemorrhages

lar exudate and the tuft cells swollen, while in others the notable features are dilatation of the capsules of Bowman with profuse hemorrhages in the periglomerular spaces.

The tubular cells are swollen, irregular, fatty, fragmented and displaced from the basement membrane (Fig. 10), with many of the collecting tubules containing red blood corpuscles, hyaline casts, granular parenchymatous cells and a few polymorphonuclear leucocytes.

Where inter- and intratubular hemorrhages are most numerous, the parenchymatous cells are loosened and necrotic.

In the case of pregnancy the parenchymatous cells showed acute widespread destruction with pale, necrotic, disintegrating substance.

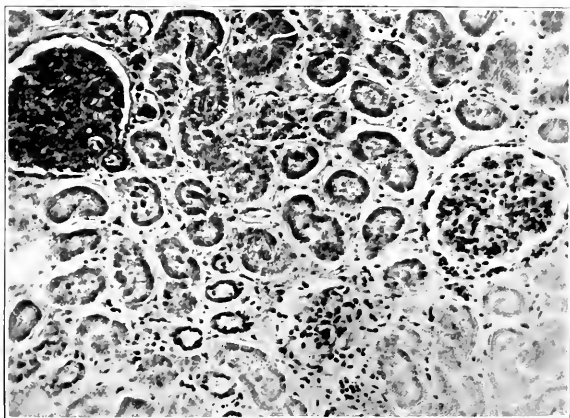


Fig. 11.—Productive nephritis with advanced acute degeneration of the flattened tubular cells.

#### Microscopic Group 2 (a):

The following is the microscopic picture of influenza generally seen in a kidney which is the seat of a diffuse productive nephritis.

In this group of cases the capsule which is already thickened by proliferated fibrous tissue contains large engorged dilated vessels, many of which are thrombotic or present hyaline changes in their walls.

Underneath the fibrous hemorrhagic capsule the vessels are thin-walled, dilated, and packed with red blood corpuscles. In patches there is fibrous obliteration of subcapsular structures in the midst of which hyaline degeneration of cell masses is a frequent feature.

In some cases the acute changes are limited to the glomeruli, in which the tuft vessels are enlarged and packed with corpuscles. Again the glomeruli may be knotted, with the tuft cells undergoing fatty degeneration, and the spaces filled with a granular hemorrhagic exudate.

Fibrous tissue has abundantly proliferated, destroying the parenchymat-

TABLE III  
ORIGINAL AND ACUTE BRAIN LESIONS

CASE NO.	ORGANIC LESIONS	SPINAL FLUID	MEMBRANES	CEREBRAL CORTEX	CENTRUM SEMIOVALE	VENTRICLES (EPENDYMA)	BRAIN STEM
1	Diffuse gliosis	Considerable	Edematous	Subi filled with fluid	Edematous	Congested	Edematous
2	Arterio-sclerotic softening	Increased	Rusting of pia mater Deeply congested	Deeply congested	Deep congestion, dilatation of spaces and acute softening	Glistening surface extended	Deep congestion and punctate hemorrhages
3	None	Marked excess	Vessels and sinuses engorged	Surfaces oozing blood—marked edema	Softened, boggy and hemorrhagic	Dilated—greatly congested	Great congestion of the substance of pons and cerebellum—basal ganglia soft and hemorrhagic
4	Sclerotic atrophy of right hemisphere	Increased	Marked congestion	Edematous, hemorrhagic plaque	Edematous	Not remarkable	Not remarkable
5	General senile atrophy	Increased	Vessels congested	Edematous	Diffuse blood pigment in all areas	Markedly congested and dilated	Numerous punctate hemorrhages through the internal capsule and gray ganglia
6	Patchy gliosis	Diminished	Slight congestion	Boggy	Moderate edema	Moderate congestion	Edematous
7	Senile atrophy	Marked excess	Basilar congestion Pial clouding	Multiple softening and hemorrhagic points	Edematous	Dilated vessels	Very edematous pons and medulla hemorrhagic
8	None	Slight excess	Marked congestion	Pale and moderately soft	Acute softening	Markedly congested	Basal ganglia notably edematous
9	Cerebral sclerosis	Marked excess	Strips easily, deeply congested and milky	Subarachnoid effusions of blood	Edematous	Dilated—ependyma congested	Diffuse congestion
10	Focal cortical atrophy	Excess	Slight congestion	Congested and presents minute hemorrhages	Edematous, few perivascular hemorrhages	Diffuse red discolorations of ependyma	Edema and congestion of all structures
11	None	Normal amount	Sinuses filled with clots, intense congestion	Very edematous Subpial hemorrhages	Dilated lymph spaces, few hemorrhages	Slightly congested	Basal ganglia show few punctate hemorrhages
12	Cerebral sclerosis	Normal	Congested Large hemorrhages	Notably edematous Few acute softening	Few acute softening	Ventricles dilated Ependyma congested	Numerous prominent punctate hemorrhages through the basal ganglia
13	Sclerotic atrophy of left hemisphere	Marked excess	Extremely congested	Very hemorrhagic and edematous	Presents numerous softening	Ventricles greatly dilated Ependyma is congested and granular	Central softening in optic thalamus
14	None	Excess	Intensely congested	Superficially anemic and edematous	Punctate hemorrhages	Ependyma reddened and distending	Softening in internal capsules and lenticular nuclei. Specimen studied with punctate hemorrhages

TABLE III—Continued  
ORIGINAL AND ACUTE BRAIN LESIONS

CASE NO.	ORGANIC BRAIN LESIONS	SPINAL FLUID	MEMBRANES	CEREBRAL CORTEX	CENTRUM SEMIOVALE	VENTRICLES (EPENDYMA)	BRAIN STEM
15	Cortical atrophy	Marked excess	Slight congestion	Thin, edematous, very few hemorrhages	Numerous hemorrhages through the occipital portion	Slight congestion of the ependyma	Pons medulla and cord show acute diffuse softening
16	Sclerotic atrophy of the left temporal lobe	Increased	Diffuse intense congestion	General edema	Very hemorrhagic	Ventricles moderately dilated, Ependyma congested, large vessels	No remarkable changes
17	Cerebral arteriosclerosis	Normal	Milky congested pia	Extensive hemorrhages and softening	Softening and hemorrhages	Normal in size	Multiple softening in the basal ganglia
18	None	Normal	Deeply congested	Superficial softening with some petechial hemorrhages	Marked edema	Ependyma dull, cloudy granular	Small area of softening in right thalamus. Small hemorrhages in internal capsules. General edema
19	None	Moderate excess	Meninges strip easily. Deeply congested	Pale, boggy, lower layers hemorrhagic, anemic surface	Not remarkable	All ependyma pale, listless	Surface congestion. Basal ganglia stained with minute hemorrhages
20	None	Normal	Congested and edematous	Thin, pale and boggy. Few punctate hemorrhages	Filled with free red blood pigment	Dark red congested ependyma	Minute hemorrhages are through the basal ganglia, the internal capsule, the pons, the medulla and spinal cord
21	Cerebral arteriosclerosis with multiple softening	Marked excess	Intensely congested. Diffusely opaque	Hemorrhagic	Hemorrhagic. Diffuse blood pigments	Ventricles dilated. Deeply congested ependyma	Basal ganglia and internal capsules show many arteriosclerotic degenerations, small free hemorrhages are numerous
22	None	Normal	Large veins with coagulated blood, membrane infiltrated with hemorrhages	Considerable free blood over the surface	Diffuse pink in color	Ependyma pink listless, choroid deeply congested	All structures soft, edematous and present punctate hemorrhages
23	None	Normal	Congestion of both dura and pia	Reddened from blood pigments	Edematous	All surfaces pale, listless and ependyma presents small hemorrhages	Basal ganglia stained by minute hemorrhages and dilated vessels
24	Cerebral arteriosclerosis	Normal	Marked congestion and subpial hemorrhages	Hemorrhagic	Edematous	Ependyma covered by bloody exudate	Minute hemorrhages through the internal capsules. Thalamus and corpus striatum are markedly edematous

TABLE III—Continued  
ORIGINAL AND ACUTE BRAIN LESIONS

CASE NO.	ORGANIC BRAIN LESIONS	SPINAL FLUID	MENINGES	CEREBRAL CORTEX	CENTRUM SEMIOVALE	EPENDYMA (EPENDYMA)	BRAIN STEM
25	None	Normal	Deep congestion of entire surface and widespread blood diffusion	Minute hemorrhages and superficial acute softening	Pink from diffused blood	Ependyma presents deep red dull surface	All capillaries of cerebellum and pons exceptionally prominent; moderate hemorrhages throughout the cerebellum, in internal capsule and corpus callosum
26	None	Marked increase	All membranes intensely congested	Deeply congested	Deeply congested	Not remarkable	Vessels of cerebellum and pons greatly dilated. Minute hemorrhages in basal ganglia and in corpus callosum
27	Parotid neurosyphilis	Marked excess	Deep congestion over all surfaces	Cloudy, edematous and hemorrhagic	Moderate blood diffusion	Ventricles present diffusely congested surface	The corpus callosum and all basal ganglia are mottled with minute hemorrhages and edematous lymph spaces
28	Syphilitic meningitis arterio-sclerotic softening of left occipital lobe	Marked excess	Intense congestion of entire surface	Presents numerous acute softening and congestion	Edematous and congested	All ventricles dilated	Basal ganglia are atrophied and hemorrhagic, acute softening through the internal capsule
29	Chronic leptomeningitis	Moderate excess	Diffuse congestion of all membranes	Cloudy and granular	Edematous	Ventricles greatly dilated, ependyma congested	The basal ganglia and internal capsules are strikingly edematous and contain many periventricular hemorrhages
30	Generalized atrophy	Marked excess	Diffusely congested	Edematous	Softening about ventricles and presents many ruptured vessels	Dilated ventricles congested	Basal ganglia extensive and congested
31	None	Slight excess	Edematous and congested	Contains numerous minute hemorrhages	Hemorrhagic	Congestion of ependyma	The basal ganglia particularly the lenticular nucleus is dotted with minute hemorrhages. Pons deeply congested
32	None	Slight excess	Marked congestion	Minute capillaries prominent	Generalized congestion and widened lymph spaces	Ependyma is pale, dull and boggy	Substances of pons and cerebellum are moderately hemorrhagic. Softened lenticular nuclei all ganglia are hemorrhagic. Corpus callosum is hemorrhagic
33	None	Moderate excess	Moderate congestion and blood diffusion	Intense congestion	Edematous and filled with punctate hemorrhages	Ependyma smooth and dull, pink in color from blood pigments	Basal ganglia filled with punctate hemorrhages substances of pons, medulla and cerebellum are congested

TABLE III—Continued  
ORIGINAL AND ACUTE BRAIN LESIONS

CASE NO.	ORGANIC BRAIN LESIONS	SPINAL FLUID	MENTINGER	CEREBRAL CORTEX	CENTRUM SEMIOVALE	VENTRICLES (EPENDYMA)	BRAIN STEM
34	Cerebral arteriosclerosis (L. V. T.)	Notable increase	Entire surface congestion	Deeply congested	Deeply congested	Dilated ventricles. Ependyma markedly brown. Blood pigments	Basal ganglia are hemorrhagic
35	Cerebral arteriosclerosis (L. V. T.) nerve atrophies	Moderately increased	Diffusely congested	Extremely thin and pale	Edematous	Ependyma diffusely red and loosened	All ganglia and internal capsules are discolored and softened
36	Cerebral arteriosclerosis (L. V. T.) with sclerotic atrophies	Enormously increased	Engorgement of vessels	Few acute softening	Hemorrhagic	Ependyma granular and congested. Ventricles widely dilated	Basal ganglia and corpus callosum with the capsules are markedly congested and present punctate hemorrhages
37	Senile atrophy	Marked increase	Marked congestion and hemorrhages	Thin, edematous and hemorrhagic	Edematous	Ependyma congested	Basal ganglia show numerous petechial hemorrhages with acute softening
38	None	Moderate increase	Intensely congested	Markedly hemorrhagic	Filled with punctate hemorrhages	Choroid plexus swollen. Ependyma covered by hemorrhagic exudate	Substance of pons, medulla and cord is filled with petechial hemorrhages. Basal ganglia edematous and hemorrhagic
39	None	Slightly increased	Diffuse congestion	Edematous, acute softening, hemorrhagic	Thickly studded with minute hemorrhages	Ependymal capillaries congested. Ependyma dull pink in color	Substance of pons and medulla and cord are hemorrhagic. Basal ganglia, pons, callosum and internal capsule are also hemorrhagic
40	Microcephalia	Normal	Vessels engorged	Edematous and pale	Hemorrhagic	Large vessels engorged	Basal ganglia acutely softened and very hemorrhagic
41	Fetus	Normal	Intensely congested. Much free blood	Hemorrhagic and soft	Softened, disintegrated	Congested	Many vessels ruptured. Substance disintegrated
42	Hydrocephalus internal	Enormous increase	Intense congestion	Petechial hemorrhages	Vessels markedly distended	Ependyma congested. Ventricles enormously enlarged	Basal ganglia flattened and distorted from pressure, but very hemorrhagic and edematous

ous structures over large areas in which hemorrhages from small ruptured vessels are seen in large numbers.

In most instances there is an acute albuminous degeneration of the living tubular cells, which are pale, bear indistinct nuclei and sometimes hyaline changes, and the lumina of the tubules contain a finely granular exudate.

The collecting tubule regions are characterized by extra- and intratubular hemorrhages, many of which cover large areas with diffusion of red blood cells; and by the acute changes in the parenchymatous cells which are greatly swollen, nearly filling the tubules, and are often indistinct without visible nuclei.

**Microscopical of Group 2 (b):** In this group of arteriosclerotic kidneys multiple small interstitial and intratubular hemorrhages are marked in the patchy sclerotic zones where congested areas seem to alternate with strips bearing normal appearing vessels.

There is a general hemorrhagic glomerulitis with much blood pigment and wide edematous spaces. The convoluted tubules present flattened, granular, cloudy cells, sometimes in stages of disintegration. (Fig. 11.) Some of the collecting tubules contain masses of red blood corpuscles, and hyaline casts are especially numerous. A few polymorphonuclear leucocytes are occasionally seen in the collecting tubule regions.

#### GLANDS OF INTERNAL SECRETIONS

**Thyroid.**—Nearly all of the adult thyroids show atrophic features and a chronic interstitial thyroiditis. Congestion with abundant deposits of blood pigment are the constant acute changes.

**Adrenals.**—These glands are usually swollen and dark colored—actual gross hemorrhages are not seen.

Microscopically there is a patchy cloudy swelling of the parenchymatous cells of the cortex, a few focal necroses, and a number of interstitial hemorrhages. The medullary portion is congested and contains many lymphocytes.

**Pituitary Glands.**—Show no remarkable acute features.

**Pancreas.**—This organ is most often swollen, congested and softened, but is occasionally pale and firm.

Microscopically all the larger vessels are engorged with blood, the capillaries prominent with congestion, and a few interstitial hemorrhages are noted. The cells of the alveolar glands and of the pancreatic ducts are in some cases irregular, shrunken, and heavily stained, while in other instances they are swollen cloudy and indistinct.

The islands are usually small, pale, and composed of a few widely separated cells. The pancreatic ducts are filled with granular debris and their walls are strikingly outlined by intensely congested capillaries.

#### CENTRAL NERVOUS SYSTEM

Table III gives the original and acute gross brain lesions.

**Meninges.** The cerebrospinal fluid is usually in moderate excess and the dura presents deeply congested vessels and engorged sinuses.

In the pia the intricate networks of capillaries are prominent over all sur-



faces of the hemispheres. The pia of the cerebrum, because of its edema, strips easily, is milky in color and is frequently opaque along the vessel sheaths.

Blood diffusion through the arachnoid meshes is seen in many specimens in which the surfaces ooze blood, where veins are dilated to enormous size, and in which free blood pigments are abundant in the depths of the sulci. In some cases the pial congestion is more pronounced over the sylvian areas, where the veins are engorged to about three times the usual size, contain solid casts of blood, and originate large hemorrhages, which extend beneath the membrane to the surrounding tissues; in others, the vessels over the frontal and parietal lobes are more prominent and contain large quantities of blood, some of which has diffused out through the arachnoid spaces; and in still other cases the bases of the frontal and temporal lobes are most intensively congested.

The gaping sulci of the senile atrophic cases are covered by cloudy membranes, which contain many bright-red capillaries, and the depths of the fissures contain much free blood, which often diffuses inward as far as the basal ganglia. The cerebellar pia is often edematous and milky-white in color, but seldom exhibits more than a slight injection of the capillaries.

*Cerebral Cortex.*—This structure usually presents a slight brownish tinge. These red pigments are deposited through the cortex and have infiltrated the white substance, having extended from the congested vessels of the numerous sulci, where in the depths this change is marked. The cortex and white matter of the convolutions are filled with edematous spaces, multiple acute softenings, and hemorrhagic points.

In some instances the parietal operculum is particularly hemorrhagic in its cortical zones. The acute changes are often very prominent, particularly softenings in both the cortex and white substance of the Islands of Reil.

#### BRAIN STEM STRUCTURES

The basal ganglia are soft, boggy, filled with petechial hemorrhages, and present a diffuse pink color. In the thalami and in the caudate nuclei the hemorrhages are somewhat larger and these structures often exhibit around the larger central vessels acute softenings which extend through to the floor of the ventricle.

The internal capsule regions contain many small hemorrhages, are darkened in color, and are extremely edematous.

The ependyma of the occipital extensions of the lateral ventricles is characterized by a bloody, slimy surface deposit, and dilated vessels. In general the ependyma of the lateral ventricles presents considerable blood diffusion, and a discolored, cloudy surface deposit composed largely of blood, cerebrospinal fluid, and loosened membranes. The large vessels of the sub-ependymal regions are always engorged with blood.

The surfaces as well as the substance of the cerebellum and pons are deeply congested and contain punctate hemorrhages, about which minute areas of necrosis are visible.

## MICROSCOPICAL CHARACTERISTICS OF THE BRAIN SECTIONS

*Frontal Lobes.*—The pia mater contains dilated thin walled, congested vessels; and large numbers of red corpuseles have poured into the enlarged fluid filled arachnoid spaces. When the membranes extend into the sulci there are deposited many red blood cells, lymphocytes, and a few plasma cells.



Fig. 12.—Rupture of cortical capillary with outpouring of blood into lymph space.

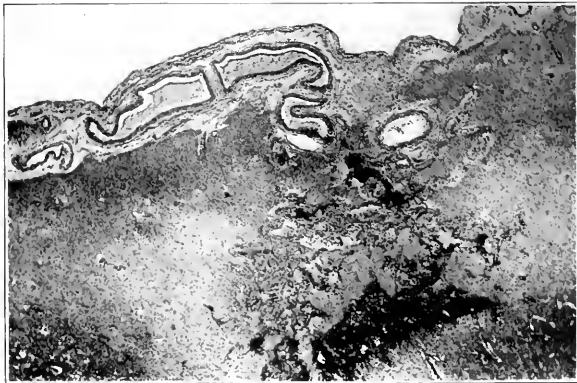


Fig. 13.—Hemorrhage into sclerotic patch in a section of frontal cortex.

The blood vessels of the cortex even to the smallest capillaries are filled with red blood corpuseles or thrombi and are surrounded by widened perivascular lymph channels many of which are filled with hemorrhages. (Fig. 12.)

Extravasations of blood into the tissues are present through the cortex and in the white matter. The central portions of these hemorrhages are necrotic

and loaded with hematogenous pigment and the periphery also exhibits a diffuse distribution of hemosiderin.

In some of the cases of arteriosclerotic brain disease patches of focal sclerosis are conspicuously prominent from hemorrhages occasioned by the rupture of the weakened walls of the new formation vessels. (Fig. 13.)

The superficial layers of nerve cells are swollen, react weakly to stains and many present an irregular areolar appearance. The deeper cell layers are imbedded in diffusely edematous surroundings; some cells are vacuolated and present varicose dendrites, a few have undergone hyaline degeneration and are surrounded by wandering cells, while others are in stages of advanced chromatolysis.

Through the white matter there is an unusual number of mononuclear leucocytes, the general picture being one of acute edema with beginning encephalitis.

*Precentral Gyrus.*—The Betz cells in general are rather pale, with an in-

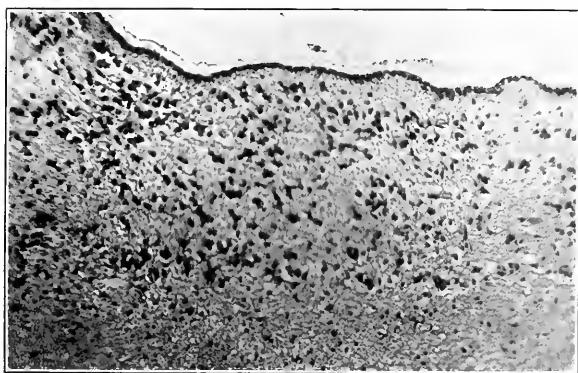


Fig. 14.—Infiltration of lymphocytes in the subependymal structures of the thalamus.

crease in pigment in the axone "hills". In some places the cells show fatty changes, hyaline alterations, and fragmentation of the chromatin granules, while in others the nuclei are heavily pigmented eccentrically situated, and in some cells showing only the nucleolus.

The smaller cortical parenchymatous cells are cloudy, nuclei indistinct and many of the smaller ones are more than half replaced by yellow globular pigmentation. In some areas these cells present fragmented chromatin marginally arranged, and showing strong basic staining properties. A few cells are fatty and elongated while others are contracted and heavily stained. The vascular changes are similar to those mentioned under the frontal lobes.

*Hippocampus.*—Punctate hemorrhages are noted in these structures, but most of the nerve cells appear exhausted. In addition to the acute changes the arteriosclerotic cases show pigmentation of the cells, some of which are completely replaced by yellow pigment, others exhibiting only central pigmen-

tation and still others with only a thin margin of original cell structure remaining. Mononuclear phagocytes are quite numerous.

*Cerebellum*.—The pia in the depths of the sulci shows a moderate lymphocyte infiltration and an engorgement of all vessels.

The molecular layer contains many lymphocytes and presents an occasional hemorrhage into the substance.

The purkinjin cells show exhaustion, fragmentation with irregular distribution of chromatin, some cloudy swelling, and numerical reduction especially along the lateral positions on the leaflets where only the faint outlines of many cells remain. In some specimens the purkinjin cells are slightly enlarged, and heavily stained, showing diffusion of chromatin through the cytoplasm.

*Thalamus*.—The overlying ependyma is usually intact, rather cloudy, and reacts weakly to stains. The subependymal structures are diffusely infiltrated with lymphocytes. (Fig. 14.)

In the brains showing advanced organic vascular changes the ependymal cells are disintegrating and the subependymal tissues present dilated perivascular lymph spaces and an occasional minute hemorrhage, about which mononuclear wandering phagocytes collect in moderate numbers.

The substance of the thalamus is edematous and all vessels are prominent from congestion. The walls of many of the larger vessels are in stages of early hyaline degeneration while others are thrombosed. A large proportion of the nerve cells appear swollen and heavily stained, but few are fragmented and in many of the older previously affected specimens, the nerve cells present the additional yellow globular pigment in the cytoplasm and an occasional pigmented nucleus.

*Lenticular Nucleus*.—There is a deep congestion of the substance, but very few hemorrhages, and no perivascular necroses are seen. The nerve cell alterations resemble those of the thalamus.

*Choroid Plexus*.—The cells of the villi evince acute hypertrophy. Numerous engorged vessels have ruptured pouring out red blood globules which are everywhere in evidence. Dilatation of the larger vessels and bright congestion of the minute capillaries are the striking features.

*Medulla*.—Many ruptured vessels are seen with surrounding zones of necrotic alterations, and accumulations of blood pigment are frequently noted near the nerve cells, which are sometimes granular, but usually are heavily stained a deep blue from the diffusion of chromatin. In many the cytoplasm has apparently burst pouring its substance out into surrounding spaces.

The capillaries of the motor regions are deeply congested and are surrounded by lymphocytes. The lymph spaces occasionally contain a few polymorphonuclear leucocytes.

*Olivæ*.—Most of the changes appear acute with intense congestion of the vessels, the walls of which are pale, weakened and ruptured producing small hemorrhages, and surrounded by lymphocytes filled with blood pigments, as are also those in the perivascular lymph spaces. Many other vessels with walls intact, but choked with blood are observed. The nerve cells have swollen nuclei, and a protoplasm which is reduced in amount and generally granular.

*Pons*.—The pons tissue shares in the general congestion and the nerve cells

of the nuclei are swollen, vacuolated and often fragmented. In many cells the chromatin presents a marginal arrangement in the cytoplasm or is occasionally massed in large pale staining lumps toward the center of the cell structure.

*Spinal Cord.*—The cord structures present an unusually intense congestion and numerous lymphocytes and plasma cells collect in the vicinity of the central canal. Some of the anterior horn cells appear fairly normal showing only a slight cloudiness, but in general the acute alterations in the nerve cells are notable. There is a tendency to clumping of the chromatin material, and in most cells there is a marked reduction in the number of nissl bodies.

#### CONCLUSIONS

1. Practically all tissues of the body presented acute changes, the result of the infection; these changes were congestion, edema, degeneration, and rupture of walls of blood vessels resulting in hemorrhages and focal necroses, and alterations in the parenchymatous cells varying in degree from simple albuminous degeneration to complete necrosis. When the organs were the seat of chronic processes having an abundance of new formation blood vessels there was a striking hemorrhagic picture produced from the rupture of these vessels, and the associated tissue reactions.

2. Mucous membranes in general exhibited one or the other of two changes, the congested vessels were plainly visible with bright capillary networks or the membranes were a diffuse beefy red color from rupture of vessels and general outpouring of red blood globules.

3. In 21 cases the kidneys were fairly free from chronic disease, but reacted strongly to the infection by acute parenchymatous cell alterations, marked general edema and universally by hemorrhages, which varied in number, size and location. In the 21 cases forming Group II of original productive nephritis, the acute changes were more diffuse and destructive, particularly the hemorrhages which were often remarkable in extent.

4. The distribution of the chronic organic brain lesions among the clinical divisions were as follows:

MENTAL DIAGNOSIS	NUMBER OF CASES	ORIGINAL LESIONS	ABSENT
Dementia Præcox	20	6	14
Arteriosclerotic Dementia	5	5	0
Senile Deterioration	5	5	0
Manic Depressive Insanity	2	2	0
Neurosyphilis	2	2	0
Undiagnosed	3	2	1
Epileptic Psychoses	2	2	0
Imbecility	2	2	0
Fetus	1	0	1
Total	42	26	16

Of the twenty cases clinically diagnosed dementia præcox, six showed original organic brain disease usually of the nature of a diffuse, or of a focal gliosis, while congestions, hemorrhages, and acute softenings were prominent through all structures regardless of the presence or absence of an original lesion.

In the brains from senile and arteriosclerotic patients presenting the usual vascular changes and lack of adequate nutrition, the acute process was

exceptionally destructive to vessel walls, and focal areas of softening were most abundant.

5. Although the above acute pathologic changes occurred in brain tissue taken from individuals with psychoses, and organic cerebral lesions, and perhaps somewhat more susceptible to further alteration, one might suppose and indeed it has been found to be the case, that these acute changes are present in varying degrees in brain tissue from those previously free from neurological and mental symptoms.

6. Among the influenzal and postinfluenzal psychoses, described by numerous writers, the acute hallucinatory disorders, depressions and dementia præcox were the most frequent. The intense meningeal and cortical edema and congestion, the acute processes in the parenchymatous cells, and the alterations in the vessel walls may account for the precipitation of many cases of acute hallucinatory disorder. In later stages of cerebral edema there has been evidence of a tendency to develop depressions, many of which are of the type indicated by the term Manic Depressive Insanity.

According to Menninger and others many cases of the dementia præcox group, and other major psychoses have passed through a febrile or postfebrile delirium and have shaded gradually into the more prolonged or permanent condition; a state which in those with a latent tendency may perhaps be determined, or aroused to activity by the cortical and subcortical necroses produced by small thromboses and ruptured vessels.

Neurasthenia with its characteristic fatigue without energy expend has been the most frequent postinfluenzal neurosis, and might also be considered in the above statement.

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## THE ALKALI RESERVE OF BLOOD PLASMA DURING ACUTE ANAPHYLACTIC SHOCK\*

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A DECREASE of the alkali reserve of blood plasma of animals during shock resulting from intravenous injection of toxic foreign proteins having been observed, a group of acute anaphylactic experiments were outlined in order to make further observations upon these changes in toxemic shock. The results observed follow.

### TECHNIC

In these experiments rabbits, dogs, and guinea pigs were used for the production of anaphylactic shock. These animals were sensitized by preliminary injections of horse serum, or purified dried egg albumen. The influence of the preliminary or sensitizing doses of protein upon the carbon dioxide capacity of the plasma was studied and found negligible. The animals, after receiving the sensitizing doses of protein, were kept upon a well balanced diet for an interval sufficient for sensitization, at the termination of which the shock experiments were made. Preliminary observations of the alkali reserve of the blood plasma were made upon every series of animals used in the experiments for several days before the production of shock, to determine the normal daily variations of the animals. Following these preliminary tests the animals were bled immediately previous to, and at regular intervals following, the injection of the shock dose of foreign protein.

### EXPERIMENTS UPON RABBITS

Eighteen rabbits were sensitized by the intravenous injection of 2 c.c., 5 c.c. and 8 c.c. of horse serum at four-day intervals. After the last sensitizing dose preliminary tests were begun to determine the daily variation of the alkali reserve in these animals. Marked daily variations in the carbon dioxide capacity occurred in the rabbits without apparent cause which made the proper interpretations of the findings in these animals questionable. The experiments were continued, however, with the following results:

Eighteen sensitized rabbits received 5 c.c. of horse serum intravenously as the toxic dose. The following table represents the average data obtained in these animals. As the findings of eighteen animals are included in the table, the results probably represent the changes which occur in the alkali reserve of rabbits during anaphylactic shock. (See Table I.)

The symptoms of acute anaphylactic shock in rabbits are not produced with any degree of uniformity and may only become manifest after several days. However, several animals included in the table showed acute symptoms. All

\*From the Department of Pathology of Manhattan Eye, Ear and Throat Hospital, and College of Physicians and Surgeons (Columbia University).

TABLE I

Average percentage carbon dioxide capacity of 18 rabbits in anaphylactic shock  
Animals received last sensitizing dose March 17, 1919

DATE		C.C. OF CO <sub>2</sub> CHEMICALLY BOUND BY 100 C.C. OF PLASMA
April 8, 1919		56.7
April 9, 1919		45.9
April 10th		59.1
April 11th—	9 A.M. before shock	54.2
	9:30 A.M.—injection 5 c.c. horse serum	—
	11 A.M.	36.3
	3 P.M.	50.2
	6 P.M.	57.3
April 12th	9 A.M.	50.3

the rabbits became emaciated and several died in four to six days. The delayed effect of anaphylactic shock in rabbits and the normal variations of the alkali reserve of the blood in these animals, with no apparent cause, render them unsatisfactory for these experiments and they were abandoned. In the above tabulated series of rabbits there appeared to exist for a brief period of time an acidosis, however, no definite conclusions are considered justifiable.

## EXPERIMENTS UPON DOGS

Several groups of dogs were sensitized to either horse serum or egg albumen. When horse serum was used each dog received 5 c.c., 7 c.c. and 10 c.c. of serum intravenously at three day intervals. When egg albumen was used, each animal received 0.25 gms., 0.5 gms. and 1 gm. of dried egg albumen intravenously at three day intervals. Neither of these substances produced a reduction of the carbon dioxide capacity of the dog plasma during the sensitizing injections. Identical results were obtained in the anaphylactic experiments, regardless of whether egg albumen or horse serum was the sensitizing protein. Therefore the results obtained are tabulated together. In the following table is shown the average carbon dioxide capacity of the plasma in twenty-one dogs, previous to and as a result of anaphylactic shock.

TABLE II

Average weight of 21 dogs, 6.5 kg.

Average amount of egg albumen to produce shock in 9 dogs of these series 0.3 gms. per kg.

Average amount of horse serum to produce shock in 12 remaining dogs, 0.5 c.c. per kg.

TIME INTERVALS	C.C. OF CO <sub>2</sub> CHEMICALLY BOUND BY 100 C.C. OF PLASMA
Before injection	52.8
15 minutes later shock dose of protein	
2 hours later	25.1
6 hours later	36.1
24 hours later	48.7

In this table is shown a great decrease of the alkali reserve of the plasma during acute anaphylactic shock in a series of 21 dogs. In several of the animals the carbon dioxide capacity dropped, in two hours' time, to 10 volume per cent. Five of the dogs died within the first six hours. Each of the five animals



showed a carbon dioxide percentage below 20 volume per cent. Two of the animals included in the table showed a drop of only 5 per cent following shock, but clinically both animals were severely shocked. In several of these dogs there was a complete restitution of the carbon dioxide capacity of the blood in six hours after the injection of the shock dose of protein, the minimum  $\text{CO}_2$  capacity remaining only a brief time. In three of the animals the blood pressure was also observed, the drop in the alkali reserve being found to begin shortly before the drop in the blood pressure, and the extent of the fall of alkali was directly proportionate to the lowered pressure. When the drop in the alkali reserve was most marked, the animals showed rapid respiration, nausea and vomiting, cyanosis, dilated pupils, and marked prostration.

Having observed a lowered carbon dioxide capacity in the blood plasma of a series of dogs in acute anaphylactic shock, it was advisable to determine how soon after the injection of the shock dose of protein the acidosis begins to develop, and to study the subsequent changes which follow in the alkali of the blood. For this study a number of animals were bled at frequent intervals after receiving the shock dose of protein. The results of these experiments are shown in Table III.

TABLE III

A dog weighing 8 kg., sensitized with 5 c.c., 7 c.c., and 10 c.c. horse serum, intravenously, April 15, 18 and 21, 1919. Upon May 13 anaphylactic shock was produced.

TIME INTERVALS	INJECTIONS	C.C. OF $\text{CO}_2$ CHEMICALLY BOUND BY 100 C.C. PLASMA
May 13th— 9 A.M.		57.6
14th— 9 "		56.7
15th— 9 "		50.0
— 9:10 "	8 c.c. horse serum intravenously	
— 9:17 "		35.7
— 9:42 "		19.6
— 9:47 "		17.6
— 10 "		
	Dog died	

This animal showed a drop in the carbon dioxide capacity of its plasma of 14.3 volume per cent in seven minutes after the injection of the toxic dose of horse serum. In this period the animal showed no noticeable, clinical symptoms of shock. In thirty-two minutes the carbon dioxide capacity had dropped to 30.4 volume per cent with the animal in profound shock. In eighteen additional minutes the animal was dead. This experiment showed a very early and rapidly progressive acidosis in acute anaphylactic shock.

The following experiment shows more clearly the progress of the acidosis after acute anaphylactic shock as the animal was bled at shorter intervals.

A dog, weighing 7.8 kg., was sensitized April 15, 18 and 21, with 5 c.c., 7 c.c. and 10 c.c. horse serum intravenously. On May 15 the dog was rendered anaphylactic. The alkali reserve of the blood was estimated two days previous to the shock to determine the stability of the carbon dioxide in this animal. In order to bleed the animal at frequent intervals a trochar and cannula was inserted into the jugular vein previous to the injection of the horse serum. (See Table IV.)

In three minutes after the shock dose of protein this dog began to show a drop in the alkali reserve of the plasma and continued to decrease rapidly for

TABLE IV

TIME INTERVALS	INJECTIONS	C.C. OF CO <sub>2</sub> CHEMICALLY BOUND BY 100 C.C. PLASMA
May 13th—9 A.M.	4 c.c. horse serum intravenously	58.3
14th—9 "		57.6
15th—9 "		55.7
—9:10 "		
—9:13 "		53.4
—9:18 "		42.1
—9:30 "		30.4
—9:45 "		28.7
—10 "		20.2
—12 "		25.2
—3 P.M.		35.2
—6 "		45.3
May 16th—9 A.M.	dog apparently well	54.8

about two hours until the dog reacted favorably to the shock, when the alkali of the blood began to increase and became normal in less than twenty-four hours. The acidosis in this animal was apparent before the development of clinical evidences of shock. In order that the frequent readings necessary to follow these changes would not influence the result of the experiment, only small quantities of blood were withdrawn and 0.5 c.c. plasma used for the tests. The proportion of plasma to the cell volume of the blood was noticeably decreased as the animal became more deeply shocked and greater quantities of blood had to be withdrawn to obtain sufficient plasma for the determinations.

## EFFECT OF ALKALINE TREATMENT

Alkaline treatment has been shown to influence favorably toxic and surgical shock, and since a marked and rapid acidosis is associated with acute anaphylactic shock, it was considered advisable to determine the effect of the preliminary administration of alkali upon this form of shock.

TABLE V

This table represents the average figures of 10 dogs.

Average dog weighed 6 kg.

Average amount horse serum given to produce shock, 1 c.c. per kg.

TIME INTERVALS	INJECTIONS	C.C. OF CO <sub>2</sub> CHEMICALLY BOUND BY 100 C.C. OF PLASMA
May 18th		55.7
19th	2 gm. sodium bicarbonate	58.6
20th	do	62.4
21st	do	67.2
22d	do	73.9
23d	do	79.7
24th—9 A.M.		80.6
10 minutes later	1 c.c. horse serum per kg.	
2 hours later		35.3
6 hours later		40.9
24 hours later		58.4

From Table V it can be seen that in dogs receiving alkaline treatment there is usually a decided drop in the carbon dioxide combining power of the serum following acute anaphylactic shock, but the minimum rarely reaches a point below 25 volume per cent, which was frequently observed in the untreated

dogs and was considered dangerous to the life of the animal. Of the ten dogs treated with alkali only one died, while in the twenty-one dogs not so treated five died. Several of the alkali-treated animals showed severe anaphylactic shock, but their recovery was rapid and their respiratory and circulatory symptoms less severe.

#### GUINEA PIG EXPERIMENTS

The beneficial effects of the administration of alkali to dogs in anaphylactic shock having been observed and as the practical application of the alkaline treatment of toxemic shock would be of considerable importance, if effective, a similar study was instituted in guinea pigs. Ninety-four guinea pigs were sensitized by the intraperitoneal injection of 0.1 c.c. horse serum. After a sensitizing period of eighteen days, these animals were used for the tests which are tabulated below.

TABLE VI

NUMBER OF GUINEA PIGS	AVERAGE (GMS) WEIGHTS	AMOUNT OF NaHCO <sub>3</sub> INJECTED	TIME BETWEEN ALKALI AND SERUM IN- JECTIONS	AMOUNT OF SERUM	PERCENTAGE MORTALITY	AVERAGE TIME BEFORE DEATH	GENERAL CHARACTER OF SYMPTOMS
10	278	none	—	0.05 c.c.	80	30 mins.	Two animals had severe symptoms of shock while others showed little or none.
10	294	none	—	0.1 c.c.	100	12 mins.	All animals had severe and typical symptoms of anaphylaxis.
10	268	none	—	0.2 c.c.	100	10 mins.	Typical symptoms of anaphylaxis.
8	287	none	—	0.3 c.c.	100	11 mins.	Typical anaphylactic deaths.
14	310	0.1 gms.	10 mins.	0.1 c.c.	85.7	17 mins.	Two animals failed to show symptoms. Twelve died.
14	285	0.3 gms.	12 mins.	0.1 c.c.	78.5	19 mins.	One animal normal. Others showed varying degrees of shock. Ten died.
14	271	0.4 gms.	20 mins.	0.1 c.c.	85.7	18 mins.	Symptoms severe. Three recovered and twelve died.
14	290	0.3 gms.	18 mins.	0.3 c.c.	92.8	14 mins.	Symptoms severe and typical but death somewhat delayed. Thirteen died.

In Table VI are shown the composite results upon the experiments of ninety-four guinea pigs, showing the effects of alkaline treatment upon acute anaphylactic shock. The table gives the average number and average weight of the animals; the amount of alkali administered; period of time intervening between the administration of the alkali and the injection of the shock dose of serum; the variations in the amount of the dose of the serum in each series of animals, to determine the approximate minimum lethal dose and the percentage mortality. The length of time necessary to produce death and the symptoms produced after the administration of the toxic dose of the protein is shown. Of the series of animals receiving 0.05 c.c. serum 80 per cent died, while the animals receiving 0.1 c.c. of serum showed a mortality of 100 per cent. The group of animals receiving 0.2 c.c. and 0.3 c.c. of serum showed 100 per cent mortality. These four groups of thirty-eight animals received no alkaline treat-

ment, being used to determine the minimum lethal dose of the foreign serum. This was found to be between 0.1 c.c. and 0.5 c.c. As 0.1 c.c. of serum killed 100 per cent of the animals, this dose was used as the amount of serum for several series of guinea pigs treated with sodium bicarbonate. Of these fourteen pigs were given 0.1 gms. sodium bicarbonate intravenously and in an average period of ten minutes, 0.1 c.c. of horse serum was injected intravenously. An average of 85.7 per cent of these animals died, showing a decreased mortality of 14.3 per cent over the untreated animals. Another group of animals received 0.3 gms. of sodium bicarbonate intravenously and in twelve minutes were given 0.1 c.c. horse serum, with a mortality of 78.5 per cent. Another group of sensitized pigs received 0.4 gms. of sodium bicarbonate and the same amount of horse serum as the previous animals, with a mortality of 85.7 per cent. A fourth series of pigs were given 0.3 gms. of alkali and 0.3 c.c. of horse serum with little protection, as 92.8 per cent died.

The results seemed to show that only slight protection is derived from the preliminary alkaline treatment of the sensitized guinea pigs in acute anaphylactic shock. The mortality of the animals remained high even if large doses of sodium bicarbonate were given.

#### CONCLUSION

1. Rabbits are unsatisfactory for the study of the changes in the carbon dioxide capacity of the plasma in acute anaphylactic shock, due to the marked normal variations from day to day in the carbon dioxide combining power of their plasma and because anaphylaxis in rabbits is very frequently not an acute shock.

2. Acute anaphylactic shock in dogs is associated with an immediate and progressive acidosis. The acidosis appears before the onset of recognizable clinical symptoms of shock. When the carbon-dioxide capacity of the blood plasma falls below 25 volume per cent the animal usually dies. The acidosis is quickly relieved after shock if the animal survives. The alkali reserve of the plasma is restored to normal in less than six hours.

3. The administration of sodium bicarbonate to dogs before anaphylactic shock has an apparent beneficial influence upon the recovery of the animals. However, it will not always prevent death even though the alkaline reserve of the plasma is restored to normal or above.

4. Alkaline treatment of guinea pigs preliminary to acute anaphylactic shock reduced the mortality 16.7 per cent in a series of forty-two animals treated with relatively large doses of sodium bicarbonate intravenously and a minimum lethal shock dose of the sensitizing protein.

## THE ETIOLOGY OF SCARLET FEVER\*

### III. THE ALKALI-PRODUCING ORGANISMS IN SCARLET FEVER

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THIS paper is a continuation of the work started in 1917 in the Bureau of Laboratories of the Detroit Health Department, and which has been briefly reported on in two previous papers.<sup>1, 2</sup>

The results of some rather intensive work in this laboratory for the past year have been so suggestive that it is felt that further report should be made, in order, if possible, to stimulate others along this line of endeavor, and to see whether or not this organism is directly connected with the disease either as the infecting agent or as an associated organism. Practically the only other reported work on the bacteriology of scarlet fever that shows any similarity to ours is that reported by Cantacuzene<sup>3</sup> in 1914. It is possible that he is working with the same organism that we are although he makes no mention of spore production which is one of the distinctive characteristics of our cultures. There are many things about these cultures that lead us to feel certain that they are not ordinary bacteria, among them being their growth on an exceedingly alkaline meat-free medium, their unusual spore production and their appearance at certain stages as revealed by the differential stains.

I think we are safe in saying that if this organism does not come into the higher bacteria group that an alkali-producing, spore-bearing coccus is something new.

However, the possibility of this organism falling into some division of the so-called higher bacteria group does not by any means exclude it as a possible cause of scarlet fever. Indeed it rather strengthens the chain because of two things we know about the disease.

A fairly regular blood picture in scarlet fever is a gradual increase in the eosinophile count usually starting after the total white cell count begins to fall toward normal. Much the same thing holds true in some other disease of nonbacterial etiology and particularly those caused by the protozoa.

Dr. V. C. Vaughan who has been making an extensive study of the epidemiology of scarlet fever called my attention to the fact that indigenous scarlet fever was practically unknown in the tropics and suggested trying lower temperatures than were ordinarily used, having in mind the work on the cultivation of certain of the protozoa particularly leishmania in kala-azar, and the trypanosomes.

The organisms which we have isolated and reported on previously, produced alkali in all sugar media. There are apparently several groups of these

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alkali-producing organisms which can be found in this disease, and we have made a rough attempt to classify them into groups according to certain definite biological characteristics.

Group one and group two have certain peculiarities which make their classification within certain limits rather easy. Since the organisms which we classify as group one have been found only in scarlet fever, we will confine ourselves very largely to a discussion of this group.

Group one. The organisms which we classify as belonging to group one are the same as the large coccus forms reported from this laboratory in previous papers. No mention has been made before of the spore production but after a varying time in subculture on ordinary media the spore production is prompt and regular. These cultures produce alkali in sugar media and are characterized by a peculiar amin odor. They have been isolated from the blood and from the throat but most frequently from the tongue. Although our number of isolations is low compared with the number of cases examined, we feel that this organism is worthy of much study because of its peculiarities. In spite of the great number of other cases examined, we have never found it in anything but scarlet fever. There are several methods which may be used in isolating this organism but two only need be mentioned.

The first is by streaking on blood agar plates and picking colonies from these. It is necessary to pick a large number of colonies since the growth on this medium is not very characteristic. These organisms grow rather slowly at first. The colonies have a regular, definite edge; are flattened and semiopaque. After a number of days on this media they tend to flatten out and become almost invisible so that in the mixed culture that we get in this way, isolation is difficult.

The second method is by the use of a modified Sabouraud's agar. The formula that has given us the best results is as follows:

Peptone	10 grams
Agar	15 grams
Sucrose	100 Grams
Water	1000 c.c.

After solution 50 c.c. of approximately normal sodium hydrate solution is added per liter and 1.66 c.c. of the stock solution of Brom-thymol blue. This stock solution is made by dissolving  $\frac{1}{10}$  gram of the dry dye in 15 c.c. of water containing 3.2 c.c. of 20th normal sodium hydrate. The hydrogen-ion value of this medium as determined by the potentiometer is about  $P_H$  9.8. Practically none of the ordinary bacteria are found to grow at all on this medium. However, we have carried cultures of this group on this medium for over a month, although growth is not as abundant or as rapid as on a less alkaline medium. Swabs from the tongue in acute cases of scarlet fever are planted on slants of this medium and incubated at 32 $\pm$ 2° C. for from two to seven days, and then kept at room temperature (approximately 25°) for about three weeks, occasionally plating on the same medium and watching for typical semiopaque blue colonies. These colonies which require two or three days to develop in good shape are transferred to slants of media of

approximately the same composition, but containing a smaller amount of alkali; the  $P_H$  value of these media being 7.4. After 24 hours' incubation at  $37\frac{1}{2}^\circ$  all the tubes are examined and any showing acid production with apparently pure culture are discarded. The alkali-producing cultures were reserved for further study and the acid tubes that showed a mixed culture were replated. By use of this method, we have been able to isolate the spore-bearing, alkali-producing organism in eight out of forty cases of scarlet fever, while material from fifty other cases including diphtheria, tuberculosis and normal people studied at the same time and under identical culture conditions failed to yield any organism of this type.

#### THE ORGANISM

The vegetating cell runs fairly uniform in size whatever media is used and whatever temperature at which the culture is grown. Growth will occur at all temperatures between  $25^\circ$  and  $42^\circ$  C. with the optimum temperature lying between  $30$  and  $40^\circ$ . The limits of size are up to five microns in diameter, and with an average size of about 3 to 4 microns. The actively growing organism is nearly round although in older cultures many apparently coccus-bacillus forms may be seen. The orientation is somewhat suggestive of the staphylococcus and in making a smear in a drop of water on a slide the organisms tend to become evenly distributed in the suspension. Spore production occurs best on blood or nutrient agar at  $37\frac{1}{2}^\circ$  C. Ordinary incubation temperature is the best whatever medium is used although spore production will occur at  $32\frac{1}{2}^\circ$  C., but is limited or lacking entirely when grown at lower temperatures. On the meat-free carbohydrate media few spores are found in comparison with the growth on richer media. The spores vary in size within wide limits and the larger ones are frequently lying free and about the same size as the organism itself. Three facts of interest stand out in the study of these spores.

First their comparatively great heat resistance. Two hours' boiling of the suspension in distilled water is not sufficient to kill; the suspension being heated in a test tube within a covered pail. We have had a few suspensions that have withstood a temperature of  $110^\circ$  in the autoclave, but in no instance have we had growth after raising the temperature to  $112^\circ$ , the autoclave being turned off when a temperature of  $112^\circ$  is indicated and allowed to cool down as quickly as possible.

Second, when cultures are made of this heated suspension there is frequently no visible growth after 24 hours in the incubator. However, if a sterile platinum wire is rubbed over the surface of the slants and the slants are returned to the incubator a heavy growth will occur in about eight hours.

Third, no spore can be demonstrated when these cultures are grown in symbiosis with other organisms. For example, the mixture of these organisms with staphylococcus after twenty-four hours on any medium tried show no spore by staining or heat testing methods.

The organism stains readily with the basic aniline dyes, but not with the acid ones. Its behavior to the Gram stain is uncertain but it probably must be classified as Gram-negative. In this connection, it is interesting that

gentian violet in concentration of 1 to 100,000 in solid media completely inhibits growth. With differential stains such as Giemsa's or some of the modifications of the Romanowsky stain in young culture, i. e., freshly isolated cultures, a beautiful contrast stain can occasionally be obtained. The ends of the organism take a deep azure color while the center area is unstained. Within this unstained area is a definite red staining body which closely resembles a nucleus, although it is possible that it is the starting of the spore.

In symbiosis with the staphylococcus, this organism is easily Gram-negative in contrast to its ordinary indifference.

The general appearance of the colony is much the same whatever medium is used. Regular edges, flat dull surface and in older cultures a flattening out and at first glance it looks as though there was no growth on the tube. In all cases there is a slight sticking to the media and the growth is somewhat viscid although nothing like that of the Friedländer pneumobacillus for instance.

The growth in broth and liquid media in general is slow. There is no pellicle formation but after two to three weeks a slimy deposit begins to form in the bottom of the tube. On Loeffler's medium growth is poor.

In litmus milk these cultures grow slowly, but after about three weeks there is a definite production of alkali. There is apparently no acidity shown at any time and no change of any importance other than the gradual production of alkali. Since these organisms grow best on the surface of the solid media, the carbohydrate work has been done in two ways, one set of cultures being planted on a modified Sabourauds agar containing peptone but no meat, and 2 per cent of the carbohydrate which we wish to test.

Another set has been grown in ordinary meat extract broth to which two per cent of the sugar has been added. In both these cases sterilization has been carried out by the intermittent method. The growth and consequently the alkali production is much more rapid on the solid media than in the fluid media.

The initial reaction of the medium is a matter of considerable importance since practically no growth occurs on media in which the  $P_H$  value is below 6.8.

We have adjusted the reaction of our media in this carbohydrate work so that after sterilization the  $P_H$  value is approximately 7. In all cases in all the sugars tested there is never any acid formation.

We have tested these cultures on the following carbohydrates: glucose, sucrose, lactose, maltose, mannite, dextrin, levulose and zylose.

The amount of alkali produced in any case is not high, the maximum amount observed being a final reaction of  $P_H$  7.8.

In this connection it is interesting to note that alkali production is more abundant and very much more rapid with sucrose than with any other sugar.

The optimum  $P_H$  value for growth of this organism is about 7.3 to 7.5 although growth will occur at values as high as 10. On media with a  $P_H$  value below 7, growth is uncertain. There is slight growth on media of the following composition: 10 per cent sucrose, 14 $\frac{1}{2}$  per cent agar, and with alkali added to bring about a reaction of  $P_H$  7.3.



To summarize somewhat the cultural characteristics of this organism the growth is strictly aerobic, optimum temperature 30 to 40, a spore-producing, alkali-producing, coccus-like organism which will grow on very alkaline media and a medium very deficient in meat or meat products.

In a great many respects this organism resembles some of the yeast family, although no budding forms have ever been observed, and multiplication as far as can be determined is by cell division.

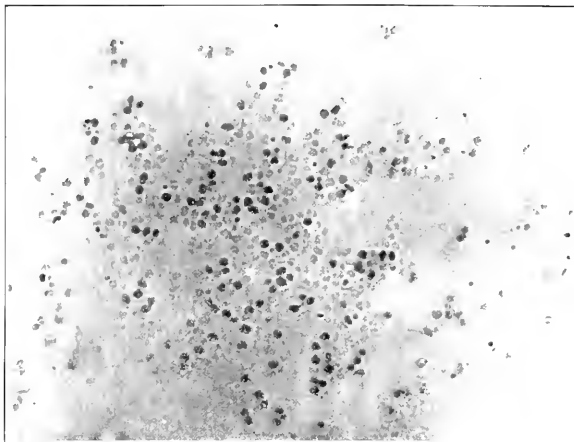


Fig. 1.—Hunteon's spore stain Group I, x1500.\*

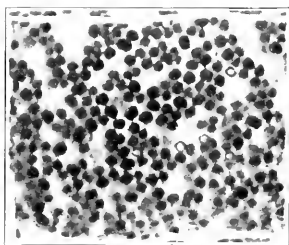


Fig. 2.—Gram's stain Group I, x1500.

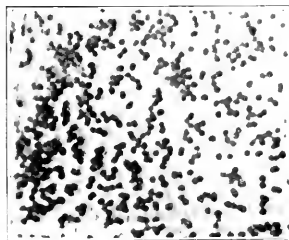


Fig. 3.—Gram's stain Group II on alkaline sacrose media, x1500.

The pathogenicity has been tested on guinea pigs, mice and rabbits. Freshly isolated cultures injected intraperitoneally into mice or guinea pigs will kill in from one to three days. Considerable evidence has been accumulated that this organism when isolated from the blood of an animal dying

\*The writer desires to express thanks to Dr. F. G. Novy, Professor of Bacteriology, University of Michigan, for these photographs.

following injection is of somewhat different morphology than is the culture injected. However, this phase of the work must wait for further investigation before a full report can be made.

The pathogenicity is soon lost and so far at least we have been unable to exalt their virulence. So far, we have been unable to demonstrate pathogenicity for rabbits either on intraperitoneal injection or intravenous injection.

#### SEROLOGY

The production of agglutinating serum in rabbits takes place rather slowly. The titer of the best serum we have obtained so far is 1 to 800. All cultures of group one are agglutinated in approximately the same dilution by this serum. Agglutinating work with the serum from convalescent scarlet

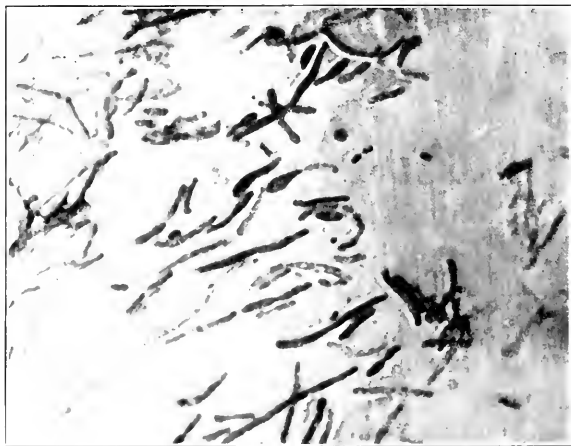


Fig. 4. Gram's stain Group 11, 8 hours on blood agar,  $\times 1500$ .

fever patients has not progressed far enough as yet to justify drawing any conclusions. There is what is seemingly a false agglutination which occurs when the diluted serum and organism suspension are mixed; and a complete report on agglutination work must wait until some later time. Group 2. The organisms which we classify as belonging to Group 2 are included in this paper because of the fact that under certain cultural conditions their morphology resembles somewhat that of group one. This culture also produces alkali in all sugars tested, but no spores have been observed. On the modified meat-free agar these organisms tend to maintain a coccus form of usually less than a micron in diameter. They are frankly Gram-negative in whatever form they may be.

The microphotograph (Fig. 3) gives a fair idea of their size and appearance when carried in culture on this medium at a temperature of  $32^{\circ}\text{C}$ .

When these cultures are transplanted from this media on to blood agar slants or plates, and these are incubated at  $37^{\circ}\text{C}$  a striking change occurs in these organisms within about eight hours. Examination at this time usually shows a tremendous increase in size and varying from a large coccus to long filaments, the largest filament that we have observed measuring 57 microns in length.

If these blood agar cultures are retained in the incubator for about 24 hours longer, the majority of the organisms have changed back again to the small coccus form and if transplanted back on to the alkali meat-free agar again show no filament.

The differential stain on these organisms in what we call the monster form show nothing very definite in the morphology or in the cellular arrangement. Multiplication in these cultures is also by cell division.

When these are carried on ordinary nutrient agar at a temperature of  $37^{\circ}\text{C}$  their general morphology resembles Group 1 so closely that mistakes might occur. The separation of Group 2 from Group 1 is fairly easy from the fact that Group 2 produces no spores and does, under the conditions outlined above, produce monster forms. The agglutinating serum prepared for members of Group 1 shows absolutely no agglutinating action when tested against group two. Group two has been isolated more frequently from scarlet fever than from other conditions, but has been found in at least one case in a normal individual.

We have several other cultures that are frank alkali producers and in general have cultural characteristics very similar to those of Group 1 and Group 2. Morphologically they are Gram-negative, rather small diplococci with which we have been unable to produce the monster forms, and in which we have been unable to demonstrate spores at any time.

There are several different organisms that in general morphology resemble Group 1 very closely. However, they can soon be ruled out by the fact that they either fail to produce alkali or else produce a colored growth and in practically every instance produce no spores. Members of these undetermined groups make up the groups of large cocci that are frequently seen in the examination of cultures for the diphtheria bacillus.

In conclusion a large coccus-shaped organism which in size resembles somewhat a yeast has been isolated from scarlet fever patients and has not as yet been found in other conditions.

This organism is characterized by alkali production in all sugars, abundant spore production and typical amine odor. The spores are very resistant to heat, withstanding at least two hours' boiling, but are usually killed by a temperature of  $110^{\circ}$  in the autoclave.

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## STUDIES ON THE RESISTANCE OF THE RED BLOOD CELLS\*

### III. THE RELATION OF CHOLESTEROL TO THE RESISTANCE OF THE RED BLOOD CELLS TO THE HEMOLYTIC ACTION OF SAPOTOXIN

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TWO theories are prevalent concerning the union of hemoglobin with the other constituents of the red blood corpuscle. The first and older theory holds that the hemoglobin is held or surrounded by a wall which is not permeable to hemoglobin. The modern theory, "Theory of Jellies," defends the belief that the hemoglobin is held in the corpuscles because of its chemical union with the stroma. Such a condition holds for all the other cells. Moreover, puncture or cross-section of the red cells does not permit of extrusion of the hemoglobin from the cell or its two halves. If the hemoglobin is set free in the corpuscles it will crystallize, showing that it must be prevented by some means from crystallizing in the normal cell. Further, the concentration of hemoglobin in the mammalian corpuscle is greater than the solubility of oxyhemoglobin in an equal bulk of water. Therefore, it seems more nearly correct to assume that the hemoglobin is maintained in a loose chemical union with the stroma of the corpuscle than to assume that it is confined within a bag or cell membrane.<sup>39</sup> Moreover, the corpuscles will not allow many of the salts to pass although they are easily penetrated by water. For instance, urea is taken up and distributed equally between the red cells and the plasma: its solution exerts no osmotic pressure upon the cells. The corpuscles behave in urea solutions of all concentrations exactly as in distilled water, that is, they give up their hemoglobin to the urea solution. Such does not occur if the urea is added to an isotonic solution of sodium chloride. Again, ammonium chloride readily penetrates the red blood cell and hemoglobin is forced into solution even if the ammonium chloride is added to isotonic saline: ammonium chloride, therefore, has a direct poisonous action upon the corpuscles.<sup>1</sup>

Certain agents are capable of liberating the hemoglobin from the red cells whether we believe that these cells are little bags containing hemoglobin or organized jellies. Such liberation of hemoglobin may result either because of physical<sup>21</sup> or chemical processes.<sup>18, 39</sup> That is, the hemoglobin may be freed from the cells by toxins such as snake poisons,<sup>9, 18, 74</sup> bacterial processes,<sup>18, 68</sup> plant poisons,<sup>18, 69, 75</sup> by small amounts of alkalis, water, ether, chloroform, salts,<sup>1, 10, 23, 24, 36, 37, 39, 40, 51, 52, 76</sup> fatty acids,<sup>41</sup> mechanical and thermal injuries,<sup>36, 39, 69</sup> and disease.<sup>11, 12, 14, 31, 32, 37, 39, 60, 63, 64</sup>

In certain respects the erythrocytes do act as semipermeable membranes. They allow water to pass in and out without changes in volume. However,

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the red blood cells are not permeable to salts. Some certain anions,  $\text{Cl}^-$ ,  $\text{CO}_3^{--}$ ,  $\text{SO}_4^{--}$ , pass in, but this can only occur providing that other ions pass out in compensation, on the other hand, the cations do not pass through at all. The quantity of  $\text{CO}_3^{--}$  ions in the erythrocytes to a marked degree influence the permeability of the cells.<sup>1, 4, 39</sup> Solutions with equal molecular concentration with the red blood cells have no power to draw water from the cells; they are therefore isotonic or possess the same osmotic pressure. Reversible osmotic phenomena can proceed for a considerable distance in either direction without injury to the cells; on this principle, a method for determining the resistance of the cells has been worked out by exposing them to a series of salt solutions of graded strength. Such a method, one may believe, determines only the osmotic resistance of the red cells. The surrendering of the coloring-matter by the red cells is not always complete in any given salt solution. This condition is explained by Hamburger<sup>24</sup> by supposing that individual corpuscles have different degrees of resistance. This observation is also true whether the cells are maintained in their normal serum or when washed. When it is recalled that the life of the red cell is comparatively short and that a certain number are being continually destroyed by the spleen and liver, it seems that such an explanation is justifiable, for the resistance of the red cells must to some degree depend upon its vitality.

The theory of membraned cells and osmotic resistance naturally fall together as interpretive measures. On the contrary the action of specific hemolytic agents, animal and vegetable poisons, cannot be easily explained by assuming osmotic changes responsible for the destruction of the cells. The stroma of the red cells freed from its hemoglobin content is toxic and causes intravascular clotting. This stroma toxicity, in all probability, is responsible, or at least leads in the hemolytic processes of certain diseases which cause a breaking down of the red cells, i. e., malaria, syphilis and hemolytic jaundice. The stroma of the normal cell, or when in combination with hemoglobin as it exists in the normal cell, is perfectly inert.

Certain vegetable glucosides such as the saponins and animal secretions (snake, insect and reptilian poisons) act as direct or perhaps specific hemolytic agents. The hemolytic action of certain bacteria is well known. In this relation it is interesting to note that the pressure of carbon dioxide acts as a protective agent to the red blood cells against the action of various hemolytic sera.<sup>65</sup> Experimental work with the saponins and anisotonic salt solutions have shown that various degrees of altered red cell resistance occurs in many of the common diseases. In cases of experimental anemia the resistance of the erythrocytes is raised to an enormous degree. For instance poisoning by phenylhydrazin so increases the resistance of the red cells that they may be placed in distilled water, even after having been washed free from the serum, without being laked. This increased resistance is associated with an increase in the stroma of the red blood cells—pachydermia.<sup>4</sup> In hemolytic icterus the resistance is low; in obstructive jaundice raised. In pernicious anemia the resistance is said to be raised while in hemoglobinuria—

paroxysmal hemoglobinuria—there is apparently no change in the osmotic resistance but a diminution of both the thermal and chemical resistance.

Various workers following Hamburger's method (anisotonic salt solutions) have reported studies upon the resistance of the red cells in various pathological conditions.<sup>19, 24, 26, 27, 37, 59, 62, 64, 72, 73</sup> The results obtained by this method may be summarized as follows. It is increased by the addition of hydrogen, nitrogen, carbon monoxide, carbon dioxide and acids, and is diminished by traces of alkalis and oxygen. Clinically it has been found that during the course of typhoid fever, pneumonia, other acute infections, pregnancy and lactation, some malignant growths, obstructive jaundice, eclampsia, diabetes, cardio-renal disturbances and secondary anemias that the isotonic tension may be increased. A lowered resistance is found in hemolytic jaundice, pernicious anemia, certain malignancies and chlorosis. (Table I).

TABLE I  
CHOLESTEROL AND THE RESISTANCE OF THE RED BLOOD CELLS TO SAPOTOXIN

CONDITION	NO.	RESISTANCE TO SAPOTOXIN			CHOLESTEROL			RESISTANCE TO SALT SOL.	
		RESIST.	HE	VARIA- TION FROM AVERAGE NORMAL	INC.	NORM.	DEC.	INC.	DEC.
Normal	185	1:13,937	91					NORM.	0.46
Pregnancy	35	1:11,271	89	+ 2,666	+	+	-	+	-
Malaria	23	1:14,380	86	- 443	-	+	-	+	+
on quinine	32	1:15,242	89	- 1,305	-	+	+	+	+
Normals	5	1:13,944	88	- 7		+	-	-	-
Tuberculosis	24	1:12,375	82	+ 1,562	+	-	-	+	-
and Lues	2	1:13,875	68	+ 62	-	?	-	-	-
and Pregnancy	2	1:10,250	85	+ 3,687	+	-	-	+	-
Typhoid	19	1:13,513	85	+ 427	+	+	+	+	+
Jaundice									
All types	31	1:12,685	79	+ 1,252	+	+	+	+	+
Obstructive	15	1:10,033	84	+ 3,904	+	-	-	+	-
Hemolytic	5	1:18,600	81	- 4,663	-	-	+	-	+
Anemia									
Pernicious	8	1:15,063	35	- 1,126	-	-	+	-	+
Cardiorenal	8	1:12,312	60	+ 1,625	+	-	-	+	-
Syphilis	7	1:15,750	58	- 1,813	-	+	+	?	?
H. Jaundice	4	1:17,125	58	- 3,188	-	-	+	-	+
Carcinoma	5	1:12,190	51	+ 1,717	+	?	-	+	-
Kidney									
Nephritis	12	1:13,958	79	- 21	+	+	-	+	+
Cardiorenal	5	1:14,500	84	- 563	-	+	+	+	+
Eclampsia	2	1:10,500	83	+ 3,437	+	-	-	+	-
Uremia	5	1:14,050	85	- 113	?	?	?	?	?
Diabetes	2	1:13,079	88	- 858	-	+	-	+	-
Malignant growths	19	1:13,500	78	+ 437	+	+	-	+	+

The above information concerning cholesterol and resistance of the red cells to anisotonic salt solutions is drawn from the literature. Our cholesterol determinations accorded with those in the literature, hence, it was not deemed necessary to tabulate these results numerically.

In previous communications we reported the results of some two thousand experiments on the resistance of the red blood cells in health and various of the common diseases to the hemolytic action of sapotoxin. The result of this work is summarized in Tables I and II. In brief our technic for making resistance determinations was as follows:<sup>24</sup> A given amount of whole

blood, 20 cmm., was exposed for five minutes at a constant temperature, 25° C., to various strengths of sapotoxin solution. Usually a set of 4 small tubes each containing 1 c.c. of the sapotoxin solution in graded dilutions was used to make the primary reading. If the point of minimal hemolysis did not occur in the first series of dilutions a set of higher or lower concentrations were tried out. The solution in which a minimal degree of hemolysis occurred was taken as the resistance point of the red cells. With this technic, the details of which appeared in our first paper, it was found that 185 determinations on normal whole blood gave an average resistance reading in a 1:13,937 sapotoxin solution. It was also shown that the removal of the serum and blood fluid from the red cells resulted in a marked loss of resistance (Table II). That is, the presence of blood fluids about the red cells in some way acted to increase red cell resistance. In disease it was found that the red cell resistance was increased in cases of secondary anemia, cardio-renal disturbances, diabetes at times, eclampsia, obstructive jaundice, some cases of malignant growths, pregnancy, some cases of pneumonia, and pulmonary tuberculosis. Red cell resistance was found diminished in cases of pernicious anemia, hemolytic jaundice, and in syphilitics on potassium iodide. The resistance of the red cells in certain cases of diabetes, some secondary anemias, malaria without quinine treatment, some cases of nephritis, lues on mercury and typhoid fever, was either normal or but slightly altered (Tables I and II).

TABLE II  
RESISTANCE OF WASHED AND UNWASHED RED BLOOD CELLS TO SAPOTOXIN

	WASHED			UNWASHED			1:1 WASHED CELLS AND SERUM	
	NO	HEMOLYSIS	Hb	NO	HEMOLYSIS	Hb	NO	HEMOLYSIS
Normal	12	1:37,375	86	185	1:13,937	90	10	1:14,050
*Syphilis	21	1:41,357	81	34	1:14,699	85	4	1:16,437
Pregnancy	5	1:37,100	87	35	1:11,271	89	3	1:12,050
Jaundice	4	1:36,750	85	31	1:12,685	80	4	1:12,564
Tuberculosis	3	1:35,000	87	24	1:12,375	81	3	1:13,025
Average	45	1:38,900	85	309	1:13,472	85	24	1:13,625

Difference between washed and unwashed cells = 25,428 points.

Table showing degree of difference in the resistance of red blood cells when washed and when in contact with their normal fluids. No, number of determinations; Hemolysis, strength of sapotoxin solution required to cause a minimal hemolysis in 5 minutes; Hb., Hemoglobin percentage.

\*Untreated.

In general our findings are in accord with the results obtained by the use of the anisotonic salt solution methods for the determination of red blood cell resistance (Table I).

In 1902 Flexner and Noguchi<sup>18</sup> published a series of experiments which throw considerable light upon the chemical nature of hemolytic actions. They found that red blood cells freed from their serum by continued washings in normal saline failed to lysis when suspended with cobra poison in an isotonic sodium chloride solution. On the other hand washed corpuscles to which a small amount of serum had been added were immediately destroyed. They also noted the same occurrence with other poisons, tetanus toxin, salanin and saponin and justly concluded that some substance was undoubtedly present

in the serum which made it possible for cobra poison to act on the hemoglobin of the corpuscles. Keyes demonstrated that lecithin is the activator of snake venoms uniting with it to form a toxic cobra-lecithin; lecithin could be substituted for serum, although lecithin alone would not act as a hemolytic agent.<sup>1, 4</sup>

The chemical function of the phospholipins is that of making, with the sterols (cholesterols), a water containing semifluid, highly reducing, auto-oxidizable, semilipoid crystalline substratum of protoplasm. They also are actively concerned in certain of the phenomena of immunity. However, the "lecithides" are very hemolytic. Lecithin emulsions have the same power to a certain degree. Hence, we may believe, that the phosphatides by combining with toxins, or possibly blood stuffs, influence the power of the latter to unite with and affect the activity of protoplasm. Either phospholipins or glycolipins have the power of binding with tetanus toxin.<sup>29</sup>

Certain metabolic products of insects, fishes, reptiles and crustaceans are also poisonous. These hemolysins of animal origin have their analogues in the saponins of vegetable origin. Lecithin favors saponin hemolysis as in snake poison,<sup>9, 32, 44</sup> while cholesterol inhibits hemolytic activity. The poisoning due to murexella is powerfully hemolytic in its effect, causing fever, jaundice, hemoglobinemia and hemoglobinuria. This poison can be made innocuous by boiling.

Recent experimental work has shown that the blood plasma and red cells are especially rich in cholesterol.<sup>3, 5, 7, 12, 15, 17, 20, 21, 22, 25, 28, 29, 30, 33, 34, 38, 42, 47, 54, 61, 62, 67, 69, 71, 80</sup> Cholesterol is found in an uncombined form in the red cells and as cholesterol esters in the plasma. Bloor,<sup>6</sup> for instance, has shown that lecithin (including cephalin) in the corpuscles is nearly double that in the plasma while cholesterol and total fatty acids are more abundant in the plasma. The amount of lecithin in the corpuscles is about double the amount of cholesterol, but in the plasma the two substances are about equally distributed. He also found that the lecithin-cholesterol ratio is constant in normal blood and in many pathological cases.

Cholesterol is one of the most important of the physiologic substances and plays a great part in certain pathologic conditions. Cholesterol forms a weak molecular union with saponaceous substances, such as digitonin, saponin, and other hemolytic glucosides. It thus protects the red blood cells by neutralization of their toxic action.<sup>1, 4, 39</sup> Red cells are continuously being acted upon by hemolyzing substances and dissolved. Hence, if the rate of destruction becomes greater than that of new formation, varying degrees of anemia must of necessity result. Cholesterol offers protection to the cells against many of these substances, cholesterol also acts to neutralize or check the action of lipolytic enzymes. Cholesterol and certain of its derivatives assist other lipins in giving to cells their power of holding large quantities of water without losing their peculiar semifluid character, and without dissolving. Cholesterol forms one of the most abundant lipins of the brain and occurs in nearly all living tissues. There is evidence to show that cholesterol is the mother substance from which the bile acids are derived; it also probably gives rise to certain lipochrom pigments and possibly to some of the odorif-



erous substances of plants and animals. The total cholesterol in the blood serum of normal individuals varies within rather narrow limits: the average amount is from 0.15 to 0.18 per cent.<sup>39</sup> A condition of hypercholesteremia may be brought about experimentally by the feeding of cholesterol or cholesterol-containing foods.<sup>16</sup> A physiologic hypercholesteremia occurs normally during the later months of pregnancy. The cholesterol content of the blood is increased in certain pathologic conditions. In complete occlusion of the common bile duct the cholesterol is high in the blood as normally in the bile. The cholesterol is increased in cases of lipemias with severe diabetes, nephritis, and arteriosclerosis in which case the esters of cholesterol form doubly refractory masses. In certain of these conditions the hypercholesterolemia appears to be associated with a mobilization of other fatty substances in the body. Local deposits of cholesterol occur in atheromatous arteries, white plaques of nephritic retinitis, arcus senilis, exanthemata, old infarcts, caseous material of tubercular lesions, and gall stones. According to Pighini the cerebro-spinal liquid of syphilites generally contains more cholesterol than normal. The blood cholesterol is usually normal or diminished in pulmonary tuberculosis, certain of the anemias, hemolytic jaundice, etc.<sup>2, 3, 6, 7, 9, 13, 14, 15, 21, 26, 35, 41, 43, 45, 47, 67, 76, 78, 79, 81</sup>

Certain acids formed by the oxidation of cholesterol are poisonous and possess a toxicity and hemolytic action comparable with some of the snake venoms. Windaus<sup>80</sup> obtained such an acid which was more hemolytic than the bile acids, and as powerful as many saponins in causing red cell destruction. Local necrosis similar to that caused by snake bite followed injections of this acid.

With these points in mind the inevitable conclusion was reached during the progress of our studies in red cell fragility that the lipins of the blood in some way determined the action of the hemolytic agent sapotoxin. As a check upon this conclusion a certain number of cholesterol and resistance tests were made upon the same bloods. In our cases the cholesterol of the blood was found high in the latter part of pregnancy, in obstructive jaundice, one case of mercury poisoning, tuberculosis, certain cardio-renal diseases, certain diabetes, eclampsia, and in some cases of anemia. Cholesterol was found low in cases of hemolytic jaundice, pneumonias associated with jaundice, in certain malignant cases; especially those associated with anemia and cachexia. In typhoid, malaria, anemias in general, syphilis with and without treatment, and tuberculosis the cholesterol findings varied. Resistance of the washed and unwashed cells gave readings comparable to those of the cholesterol content of the blood (Tables I and II). In the one case of chronic mercury poisoning it was impossible to wash the red cells without causing their complete destruction. However the cholesterol content of the blood was found to be 285. Apparently, in this case the high content of cholesterol of the serum alone was sufficient to prevent breaking down of the red cells. A similar condition was frequently observed while working with syphilitic bloods, especially with those which had been treated with mercurials. Our

findings relative to the cholesterol of the blood are comparable to those reported by other workers for similar diseases.

As previously shown<sup>21</sup> the removal of the blood fluids from the red cells results in a marked lowering of the resistance of the red cells to sapotoxin solutions. However the same washed cells diluted 1:1 with their own serum demonstrated a practically normal resistance. Similar observations were made on diseased bloods the results of which are shown in Table II. Numerous observers have shown that the blood fluids contain cholesterol. Hence, if as has been assumed the cholesterol content of the blood fluids acts as a protective agent against the hemolytic action of sapotoxin, then variations in the cholesterol content of the blood fluids about the cells should result in variations in the resistance of the red cells. That such a condition does subside is shown by the following considerations.

The red cells from 4 cases of obstructive jaundice when washed gave an average resistance of 1:36,750. The average resistance of 12 determinations made on normal washed cells was 1:37,375. However the red cells in whole blood show a great degree of resistance in cases of obstructive jaundice—1:10,033. Normal washed cells diluted with their own serum and cells in whole blood show a normal degree of resistance—1:13,937. In cases of obstructive jaundice the cholesterol of the blood fluids is increased,<sup>21, 25, 47, 61, 62, 77</sup> hence if the cholesterol content of the serum acts as an antihemolytic agent then a serum containing a high cholesterol content should raise the point of resistance of normal red cells when diluted with such a serum and exposed to the action of sapotoxin. That such really occurs is shown by the following experiment upon two jaundiced cases: Case X, catarrhal jaundice of mild degree and short standing, gave an average resistance of whole blood of 1:12,750. The red cells when freed from their blood fluids gave an average resistance of 1:38,000. The resistance of the same cells when diluted 1:1 with their own serum gave an average resistance of 1:13,750. The cholesterol content of the blood was 168. Case Y, obstructive jaundice of long standing, gave an average whole blood resistance of 1:9,000. The washed cells gave an average resistance of 1:34,000. A dilution of the washed cells with their own serum gave a resistance reading of 1:11,500. The cholesterol content was 292. Dilution of washed cells of Case X with the serum of Case Y which was high in cholesterol content gave an average reading of 1:10,750, an increase of 3000 points in the resistance of the washed cells when surrounded with their own serum. On the other hand, a 1:1 dilution of the red cells of case Y with the serum of Case X, which was lower in cholesterol content, gave an average reading of 1:17,500, or a loss of 6,000 points in the resistance of the cells to sapotoxin. The results of this experiment show that not only the blood fluids as such but also their cholesterol content act in such a manner as to raise the red cell resistance. This protection offered the red cells by cholesterol is, no doubt, of a chemical nature. That is, the hemolytic power of sapotoxin becomes manifest in proportion to the degree of neutralization of its action by cholesterol.<sup>2, 22, 39, 48</sup>

The above considerations lead to the assumption that the increased cholesterol content of the blood in cases of obstructive jaundice is at least in great part responsible for the increased resistance of the red cells to hemolytic substances. This assumption becomes weightier when it is recalled that cases of hemolytic and congenital jaundice show a reduction of the blood cholesterol and also a decreased resistance to the action of anisotonic salt solutions<sup>11</sup> and sapotoxin.

In the anemias Bloor<sup>6</sup> has shown that whenever the percentage of the blood corpuscles drops below one-half of the normal that abnormalities appear in the lipid content which in the order of their magnitude and occurrence are: (a) high fat in plasma; (b) low cholesterol in plasma and occasionally in the corpuscles; (c) low lecithin in the plasma. Such observations offer no certain evidence that abnormalities in the blood lipoids are responsible for anemia, however, the low value for cholesterol which is an antihemolytic substance, and the high fat fraction which may indicate the presence of abnormal amounts of hemolytic lipoids in the blood may be considered as possible causative factors in the production of anemia.<sup>4, 6, 15, 26, 41, 43, 53, 57</sup> Whether a disturbed lipid metabolism is the cause of anemia or not, the fact remains that the resistance of the red blood cells varies in proportion to the cholesterol content. Hence, it may be assumed that a normal cholesterol content is essential to the maintenance of the normal resistance of the red cells to hemolytic substances.

#### SUMMARY AND CONCLUSIONS

The present work, therefore, leads to the conclusion that cholesterol is an important element in the protection of the red cells against the hemolytic action of sapotoxin. Because of this conclusion we feel that a chemical examination of the blood for cholesterol is to be greatly preferred to the more laborious and inaccurate task of estimating the red cell resistance to hemolytic agents. Furthermore, we would emphasize the fact that resistance determinations are of little clinical value save for the differentiation of the two types of jaundice because of the wide range of variations that occur in many of the common diseases.

In brief, our results are as follows: The red cells may be considered as composed of jellies which exist and depend upon their content and peculiar behavior of lipins. The cholesterol, both of the cell itself and about the cell acts as an antihemolytic while lecithin tends to combine with toxins to form lecithides. Such formations react upon the cell to destroy its composition, thereby liberating the hemoglobin from the stroma. Diseases in which the cholesterol content is high show an increased red cell resistance to sapotoxin solutions; those showing a low cholesterol content also show a lessened degree of resistance to specific hemolytic agents. Hence it may be concluded that the cholesterol of the blood in great part determines the degree of resistance of the red cells to sapotoxin solutions.

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# LABORATORY METHODS

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## THE WASSERMANN TEST AND ITS INTERPRETATION

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### I.

#### INTRODUCTION

JUDGING from the requests for information regarding the Wassermann test which come to this laboratory, it was felt that a paper embracing, first the requirements of the laboratory for correct tests; second, the responsibility of the physician toward this end, and third, the interpretation of the test, would be helpful to many who are concerned in venereal disease control. It was with this view in mind that this paper was prepared.

Many attempts have been made to standardize the Wassermann test. The New York City Department of Health,<sup>1</sup> for example, inaugurated a movement to this effect among New York City Wassermann workers in 1915. It was soon found, however, that there existed so much difference of opinion among the different workers regarding the procedure of the test, that the attempt was finally abandoned. Standard Wassermann procedures have also been attempted by the U. S. Public Health Service<sup>2</sup> and the Massachusetts State Board of Health.<sup>3†</sup> It appears that the standardization of the Wassermann technic, to the extent of its being employed by most Wassermann workers, is a very difficult task. The standardization of the essential requirements of the test without regard to the procedure, however, can be brought about.

This phase of standardization was discussed in a recent paper from this laboratory<sup>4</sup> where a number of requirements were set down as being essential for correct Wassermann tests. These requirements, as well as others affecting the correctness of this test, will be more fully considered below. There are also phases influencing Wassermann results which are largely in the hands of physicians and health officers. Finally, there are numerous questions relative to the interpretation of Wassermann results. Each of these, for the sake of clearness, will be discussed under separate headings.

### II

#### WHAT THE STATE LABORATORY IS DOING TO INSURE CORRECT WASSERMANN TESTS

1. *The Duplication of Wassermann Tests.*—It is evident that the Wassermann test should be performed with the highest accuracy. This particularly applies to the public health laboratory. In a hospital laboratory, if the Was-

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<sup>†</sup>The Studies on the Standardization of the Wassermann Test by Kohner and Co-workers (Am. Jour. Syph., 1919:21) have not been completed at the time of preparation of this paper.

sermann results do not check the clinical diagnosis, there is no serious harm; another specimen can be obtained with little difficulty and the test repeated. In a public health laboratory, however, where the specimens come from physicians who are scattered over a wide area, the Wassermann results must necessarily be accepted as practically final. For a public health laboratory to report a positive Wassermann on one free from syphilis is a very grave error indeed. Then again, to report a false negative might result in seriously endangering the health of the community. To overcome both of these possibilities, this laboratory runs two Wassermann tests of varying sensitiveness on every specimen coming for examination. One of these tests is carried out with the alcoholic-extract antigen and ice-box fixation. This procedure is generally accepted to be free from the possibility of picking up false positives. There are, however, isolated cases where the cholesterinized antigen appears to be more sensitive than the alcoholic-extract antigen. A duplicate series of tests are thus carried out with this antigen in order to pick up the occasional weak positive which the alcoholic antigen might miss. And in order to help overcome the tendency of the cholesterinized antigen to pick up an occasional false positive, only three antigenic units are employed, also, instead of ice-box fixation, water-bath fixation is resorted to. The interesting fact regarding these two procedures is that they check practically 100 per cent. Of 8000 specimens examined during the past four months, only five did not check by both methods, or one specimen in 1600.

The employment of two distinct Wassermann procedures of varying sensitiveness for each specimen of blood, in our opinion, goes a long way toward the solution of the problem of correct Wassermann tests. The mere duplication of tests, however, does not necessarily mean that the final results are absolutely correct. What the State Laboratory is doing to render the results of the *individual* Wassermann tests of the highest accuracy, will now be considered.

2. *The Removal of Antishcep Amboceptor from the Patient's Serum.*—Perhaps the main reason why natural amboceptor is not removed from the patient's serum in a large number of Wassermann Laboratories, is because of the tediousness of the task.<sup>7</sup> Prof. Charles E. Simon,<sup>8</sup> a strong advocate of the removal of natural amboceptor from the patient's serum, makes this significant statement regarding this question in a recent paper: "I have reason to assume that there is a tendency to let well enough alone, on the ground that the original technic is time-consuming enough, as it is, and that any tendency to further lengthen the process would be disregarded."

The fact that natural amboceptor introduces a definite source of error in the Wassermann test is well established.<sup>5-8</sup> If the blood of a syphilitic patient should happen to contain a small number of so-called syphilitic antibodies and a relatively large amount of natural amboceptor, it would be likely to give a negative Wassermann result. Thus positive reactions are continuously apt to be lost, if the natural amboceptor is not removed from the serum.

In this laboratory, the removal of antishcep amboceptor from the patient's serum is part of the routine procedure of the Wassermann test. Our method is fully described in another paper.<sup>9</sup> Briefly, it consists of adding concentrated



sheep-cells to inactivated sera in the proportion of 1 drop to 1 c.c. and permitting it to extract for 10 minutes at room temperature. The sheep-cells are then thrown down by centrifuging at high speed and the clear supernatant serum is ready for use. This simple procedure for removing natural amboceptor has been applied in this laboratory to about 16,000 Wassermann tests, with entirely satisfactory results. It is efficient in its amboceptor removal; it does not cause the serum to become anticomplementary; and its time-consuming element is practically negligible.

3. *Complement and Amboceptor and their Daily Titrations.*—Complement is obtained by bleeding guinea pigs under anesthesia directly from the heart. The blood is placed in the ice box immediately after clotting and the clear serum is drawn off about 15 hours after bleeding. From 3 to 5 pigs are bled daily in order to insure the uniformity of the complement. No complement is employed unless it is practically free from natural amboceptor and possesses a good complement titre.

The amboceptor employed is prepared by immunizing rabbits with sheep-cells, previously washed five times with saline. Healthy rabbits are given three intravenous injections of packed cells at 48-hour intervals. The first injection consists of 1 c.c. and the second and third of 2 c.c. each. Four or five days after the last injection, an amboceptor titre of about 1-2000 is usually obtained.

The sheep cells are obtained once every three days from our own sheep kept for this purpose. In this regard, our advantage over those workers who are obliged to obtain sheep blood from the abattoir is evident. In the latter case, the resistance of the corpuscles to hemolysis is likely to vary from day to day, while in our case this factor is kept practically constant.

The cells are washed four times with salt solution in order to wash them free from serum; and after the fourth, or final washing, they are packed by centrifugation at the same speed for an equal period of time every day. The concentration of cells is in this way kept uniform from day to day. After the cell suspension is made up in its proper dilution for the day, it is filtered through several layers of cheesecloth. This is done as a special precautionary measure in order to remove tiny clots which frequently find their way in the suspension, and which have a tendency to absorb large amounts of amboceptor.

The unit of new amboceptor is determined by preparing a series of dilutions of amboceptor-serum and titrating each with 0.1 c.c. of a 5 per cent suspension of sheep-cells and 0.1 c.c. of 1-10 pooled guinea pig complement (1-10 quantities of classical Wassermann). The dilution of amboceptor-serum aimed at, is one which contains two units of amboceptor in 0.1 c.c. This dilution being determined, it is titrated daily for a week with different pooled complements, in order to establish a permanent amboceptor unit.

Complement is titrated daily to determine the smallest amount which will hemolyze the standard quantity of sheep-cells in the presence of 2 units of amboceptor. This smallest amount is known as the complement unit, and the obtaining of this unit is considered of such importance that it is not left for one worker to determine it. Two workers make complement titrations

and determine the complement unit independently of each other, and these units must check or the titrations are repeated.

The problem of complement fixation, as the name implies, is the problem of the amount of complement "fixed" or "bound" by serum and antigen. This amount, as is well known, is far from being unlimited. An excess of complement can easily obscure the amount fixed by serum and antigen and render a syphilitic serum negative. An insufficient amount of complement, on the other hand, can render a negative serum positive. It is felt, therefore, that the determination of the complement unit with the utmost care is time entirely well spent.

We do not, however, stop with daily complement titrations; we daily titrate our amboceptor as well. This is done not because of the slight changes in the keeping quality of the amboceptor, but in order to insure that no less than 2 units of amboceptor will be employed with the complement and cell suspension of any given day. These amboceptor titrations are carried out toward the end of the fixation periods—just before adding amboceptor and cells to the tests. And as a result of these extra titrations, we have been able to avoid the occasional difficulties which come up in a Wassermann laboratory, due to an unbalanced hemolytic system.

The advantages derived from these daily amboceptor titrations are fully discussed in another paper.<sup>10</sup> Suffice it to say in this connection that these titrations enable one to determine the amount of complement which has deteriorated during the fixation period. We employ 2 units of complement in our tests, but by the end of the fixation period, when amboceptor and cells are ready to be added, we might be dealing with  $1\frac{1}{2}$  to  $1\frac{1}{4}$  complement units instead. In other words, the complement potency might have been lowered sufficiently to actually affect the balance of the hemolytic system. Whenever this happens, the amboceptor titrated against the 2 units of complement employed in the test, is considerably weaker than it should be and is adjusted accordingly.

4. *The Antigens and their Daily Titrations.*—Two antigens obtained from different sources are employed: an alcoholic extract of beef-heart and a cholesterinized antigen of pig-heart. The alcoholic antigen is prepared according to Newman and Gager,<sup>11</sup> who recently developed this method at the Johns Hopkins Hospital. The beef-heart is first freed from fat, fiber and blood-vessels and is then ground and dried. The dried beef-heart is then extracted several times with ether, after which it is extracted with 95 per cent alcohol. The ether extract possesses little antigenic value, is rich in anticomplementary and hemolytic substances, and is discarded. The subsequent alcoholic extract, on the other hand, possesses an unusually high antigenic value with comparatively little anticomplementary and hemolytic properties.

The pig-heart antigen is prepared in the same way as the beef-heart antigen, except that it is half saturated with cholesterol, or what is equal to the same thing, 0.1 gm. of cholesterol is added to every 100 c.c. of the alcoholic antigen.

What is no less important than the preparation of the antigens is their standardization; that is, the determination of the anticomplementary and hemolytic properties and, finally, their antigenic strength or complement-binding

power. The smallest quantity of antigen, which will give a 4-plus with a known syphilitic serum, is spoken of as the antigenic unit, and because of the different properties of the two antigens employed, the respective antigenic units as used in the tests, vary widely. Thus, while the alcoholic extract antigen in present use contains from 8 to 10 antigenic units, that is, from 8 to 10 times the quantity which will bind complement with a 4-plus serum, the cholesterol antigen employed contains but 2 to 3 antigenic units or only 2 to 3 times the quantity which will give a 4-plus with a known syphilitic serum. Experience has shown that, while the alcoholic antigen possesses no tendency to pick up false positives, the cholesterol antigen possesses this property to a considerable degree. Therefore, by employing but 2 or 3 antigenic units of the cholesterol antigen in the test, it is practically impossible to lose positives and at the same time unlikely to pick up false positives. The standardization of new antigens is carried out daily for a week with different pooled positive sera, and these antigens are finally employed in the tests only after the antigenic unit is sufficiently removed from the anticomplementary and hemolytic units.

Double antigen controls are employed with each series of nine Wassermann tests. Besides this, however, in order to further insure the constancy of the binding power of the antigens, a daily titration of each antigen is run with the tests. These titrations, aside from giving the antigenic strength with the daily hemolytic system (amboceptor, complement and cells), insure also this prerequisite, that at least four times the quantity of antigen used in the test is not hemolytic and does not by itself bind complement, that is, is not anticomplementary.

With regards to fixation, it might be added that two different modes are adhered to: Ice-box fixation for a period of 4 hours, in the case of the alcoholic antigen, and water-bath fixation for  $1\frac{1}{2}$  hour in the case of the cholesterol antigen. The superiority of the ice-box method of fixation over water-bath fixation, when the alcoholic extract antigen is employed, is well established. McNeil<sup>12</sup> was the first observer in this country to show the importance of ice-box fixation, and his technic is still adhered to in the New York City Department of Health. Ottenberg,<sup>9</sup> McNeal and Smith,<sup>13</sup> and others<sup>14</sup> have since pointed out the efficacy of this form of fixation.

The fixation of the cholesterolized antigen is carried out in the water-bath at 37.5° C. for  $1\frac{1}{2}$  hour. The employment of ice-box fixation with cholesterolized antigen means the picking up of large numbers of false positives, as has been pointed out by a large number of workers.<sup>13, 14</sup> On the other hand, the fixation with this antigen for  $1\frac{1}{2}$  hour in the water-bath, reduces this tendency to pick up false positives, to a minimum.\*

5. *The Daily Control Systems.*—When the various materials which enter into the Wassermann test are prepared with the utmost care, the performance of the test itself becomes a relatively simple matter. It will be recalled that the underlying problem in the Wassermann test is to find whether some unknown serum in combination with the proper antigen will bind complement and thus prevent hemolysis of a standard amount of corpuscles in the presence of their

\*Since the preparation of this paper we have been employing a fixation period of 1 hour at ice-box temperature with the cholesterolized antigen tests, with excellent results. See forthcoming issue of *Arch. of Dermat. and Syph.*

specific amboceptor (hemolysin). And, if properly controlled, no undue difficulties ought to present themselves.

The first step in the test is the standardization of the hemolytic system, that is, the finding of the proper amounts of complement and amboceptor necessary to hemolyze a standard amount of sheep's corpuscles. This being accomplished, it must be proved that the patient's serum alone, and antigen alone, do not in any way interfere with hemolysis. Then, if the patient's serum in combination with antigen prevents hemolysis, the test is positive; if they do not prevent hemolysis, the test is negative.

Not infrequently, serum and antigen will cause partial hemolysis. When this happens, the results are interpreted in accordance with the degree of hemolysis, employing the Citron<sup>15</sup> classification.

No Hemolysis or complete fixation = 4+ + +

25% Hemolysis or 75% fixation = 3+ +

50% Hemolysis or 50% fixation = 2+ +

75% Hemolysis or 25% fixation = +

87½% Hemolysis or 12½% fixation = ±

100% Hemolysis or no fixation = -

Superimposed on all the precautionary measures mentioned in this paper, daily control systems are employed with each of the tests, which are a source of great help to us. Aside from running a 4-plus and negative control, we also run a doubtful control. As is seen from the outline, a doubtful reaction is somewhere between a one-plus and a negative, representing about 12½ per cent of complement binding; and this doubtful control is expected to show approximately the same amount of complement fixation from day to day.

The appearance of these doubtful controls often gives us a clue as to how some of the "borderline" tests are to be read. Thus, if on a given day the doubtful control appears to be more like a one-plus than doubtful, we consider our system slightly "slow", and if on that day, we are in doubt as to whether to read a given test two-plus, or three-plus, we read it two-plus. If, on the other hand, our doubtful control is very weak, we consider our system slightly "fast", and we read our borderline tests accordingly.

In conclusion, it might be stated that the Wassermann test being performed with biologic extracts and fluids, can hardly be expected to behave with the same exactness as a purely chemical test. This, in our opinion, is the very reason why no effort should be spared in rendering this test as accurate and reliable as its inherent biologic factors will permit. With every step of the test carefully controlled, it is felt that a high degree of precision is being attained. What particularly encourages us in this effort is the fact that our two Wassermann systems check practically 100 per cent.

### III

#### WHAT THE PHYSICIAN AND HEALTH OFFICER CAN DO TO HELP INSURE CORRECT WASSERMANN TESTS

1. *The Prevention of Hemolyzed Specimens.* Eighty specimens a month, on an average, reach this laboratory in a hemolyzed condition. Yet the waste

of time these specimens cause the physician, the patient and the laboratory can, in most cases, be prevented.

There are many reasons why blood specimens hemolyze. According to Olson<sup>16</sup> hemolysis is due largely to the employment of the syringe. This author claims that the force required in discharging the blood from the syringe into the vial breaks the fragile corpuscles. Roderick,<sup>17</sup> however, is inclined to disagree with this worker, maintaining that he has used the syringe method of blood collection in 14,500 cases with no more than 2 per cent hemolyzed specimens. It would appear that even 2 per cent is an unnecessary waste, and, if at all possible, ought to be avoided.

By far the largest number of hemolyzed specimens is caused, in our opinion, by the fact that proper clotting is interfered with. The clumping together of the red cells during coagulation tends to preserve these cells, thereby keeping the serum practically free from hemolyzed corpuscles. The more perfect the clot, the less chance there is for hemolysis. While the less perfect the clot, the greater chance for hemolysis. Recently a physician brought to the laboratory a specimen of blood which he had drawn only ten minutes before. This specimen showed marked hemolysis—undoubtedly because the blood was continuously shaken and proper clotting interfered with.

Cold retards coagulation. It is therefore best not to place the blood vial in the ice box until after the blood has coagulated. Lack of air, also, retards coagulation. This factor is partially overcome by slanting the blood vial and thus giving the blood a larger surface exposure.

It is assumed that whatever procedure is employed in obtaining blood, that sterile conditions are adhered to, and that the syringe and vial are dry and clean. If this is done and the vial slanted and permitted to remain at room temperature until clotted, the specimen ought to reach the laboratory free from hemolysis, even though it may be several days en route.

Needless to say that the best way to prevent hemolysis of Wassermann specimens is to forward corpuscle-free serum to the laboratory. This can easily be accomplished by centrifuging the blood specimen and drawing off the serum by means of a capillary pipette. When a centrifuge is not available, clear serum can be obtained by breaking up the clot with a wooden applicator and placing the specimen in the ice box for several hours. It is particularly advisable to send serum to the laboratory during hot weather when there is a greater tendency for the blood to undergo decomposition.

2. *The Prevention of Anticomplementary Specimens.*—It will be recalled that a serum is anticomplementary when it is by itself, without antigen, capable of absorbing or "fixing" unusually large amounts of complement. Thus, while an average serum, in the quantity employed in the test, will absorb from  $\frac{1}{10}$  to  $\frac{1}{2}$  of a unit of complement, an anticomplementary serum in the same quantity may absorb from 2 to 10 of this reagent.

Theoretically, a Wassermann test can be run with an anticomplementary specimen with reasonable accuracy. The first step is to determine the number of units of complement that the anticomplementary serum will absorb. This being accomplished, the second step is to find out how much more complement this

serum will absorb, when mixed with a standard amount of Wassermann antigen. To illustrate: If a given serum absorbs, let us say, 6 units of complement by itself and 8 or more units of complement when mixed with antigen, it is reasonably safe to consider the serum as giving a positive Wassermann reaction. The reason it is not well to run Wassermanns with anticomplementary specimens is that the results are *reasonably* correct, but not *absolutely* correct. And, until we learn more about the chemical nature of anticomplementary specimens, it is far better to obtain another specimen from the same patient and perform a regular Wassermann test.

Why are specimens anticomplementary? Frequently because of bacterial contamination. Craig<sup>18</sup> found that, if sera are inoculated with staphylococcus aureus and albus and other common organisms, anticomplementary properties are developed in practically all cases. This investigator further found that such contaminated specimens are actually liable to give false-positive results.

Occasionally, blood specimens which are known to be free from contamination are, nevertheless, anticomplementary. There is not, to our knowledge, a scientific explanation for this phenomenon.

When one recalls, however, that in most cases, anticomplementary specimens are due to bacterial contamination, it is evident that we are dealing with a condition which is easily preventable. Indeed, there is not sufficient reason why blood specimens should ever be contaminated, considering particularly the marked inherent germicidal properties of freshly drawn blood.

3. *Factors Influencing the Wassermann Test.*—Considering that the biology of the Wassermann test is as yet unknown, it is evident that every factor which might influence the correctness of the test should be noted. Thus, blood taken during the height of the febrile period in any of the febrile diseases will frequently give a positive Wassermann reaction in patients apparently free from syphilis.<sup>19</sup> The ingestion of alcohol appears to have an opposite effect. Within twenty-four hours after taking some alcohol, positive reactions will frequently become negative. This, it appears, does not apply to gin. Indeed, it is claimed that within twenty-four hours after the intake of gin, a normal individual will occasionally give a positive Wassermann reaction (Wile). Blood for a Wassermann test taken during anesthesia will also frequently give a false-positive reaction.

Among the nonsyphilitic diseases which have been reported to give a positive Wassermann reaction, are frambesia (yaws), leprosy, the febrile stage of malaria, relapsing fever, yellow fever, and occasionally pellagra. Fortunately, with the exception of malaria, most of these diseases are quite uncommon and, therefore, play but a little part in influencing the interpretation of the test.

4. *The Handling of Wassermann Specimens.*—The physician, who sends specimens to the laboratory to be tested, has a right to expect that they will be looked after and examined with the greatest care. Has not the laboratory the same right to expect similar care on the part of the physician in the proper taking and forwarding of specimens?

Blood for Wassermann tests reaches this laboratory in bottles of nearly every conceivable type. It is assumed, of course, that in practically all cases

they have been properly washed and sterilized before using. The occasional container, however, which is hastily washed, may hold chemical substances that might influence the correctness of the Wassermann test. In order to overcome just such conditions, this Health Department Laboratory, as well as similar laboratories, furnishes sterile vials upon request, and as far as possible only these vials should be used.

Health officers who are called upon to obtain specimens of blood in large numbers, as is frequently true in testing food-handlers, are particularly urged to see that those who record the names are sufficiently responsible to recognize the great danger of confusing specimens. An error of this kind recently came to our attention and would have worked serious harm, were it not for the fact that the health officer traced and corrected it in time. There is but one way to overcome such errors, namely, that all those who are in any way connected with Wassermann specimens must be taught the importance of handling such specimens with the utmost care.

#### IV

##### THE INTERPRETATION OF WASSERMANN RESULTS

1. *Interpreting the Laboratory Report.*—Some laboratories report results of the Wassermann test as positive, doubtful and negative. Other laboratories report Wassermann results as two-plus ( $++$ ), one-plus ( $+$ ), doubtful ( $\pm$ ), and negative ( $-$ ). Still others report four-plus ( $++++$ ), three-plus ( $---$ ), two-plus ( $--$ ), one-plus ( $-$ ), doubtful ( $\pm$ ), and negative ( $-$ ). This last mode of reporting as proposed by Citron is adhered to in this laboratory.

A four-plus ( $++++$ ) report means that the Wassermann test is 100 per cent positive; a three-plus ( $---$ ), that it is 75 per cent positive. A two-plus ( $--$ ) report means that the test is 50 per cent positive; a one-plus ( $+$ ), that it is 25 per cent positive; and a plus-minus ( $\pm$ ), that it is from 10 to 15 per cent positive. We adhere to this mode of reporting, because in our opinion it is of greater help to the physician in interpreting his clinical findings.

We are frequently asked to interpret the meaning of a doubtful-positive or plus-minus ( $\pm$ ) report. A doubtful-positive is the weakest positive reported, ranging as was stated above, from a 10 to a 15 per cent positive Wassermann test. In the absence of clinical history and findings, such a report could undoubtedly be disregarded. In the presence of clinical manifestations, however, a doubtful report should be considered as a weak-positive reaction.

The Wassermann tests performed with the cholesterinized antigen show a tendency of "picking up" doubtful positives, even in normal individuals. The tests performed with the alcoholic antigen do not appear to possess this property. Under these conditions, when a Wassermann test is reported doubtful-positive ( $\pm$ ) with the cholesterinized antigen alone, it is far less significant than when the test is reported doubtful-positive ( $\pm$ ) with both the alcoholic and cholesterinized antigens, or even with the alcoholic antigen alone.

When the clinical manifestations on the part of the patient are of a doubtful nature and the Wassermann report is doubtful positive ( $\pm$ ), it is well to repeat the Wassermann test two or three times at about 10-day intervals. The

repeated tests may be negative, doubtful-positive ( $\pm$ ), or one-plus (-) or two-plus (++) . The clinician will thus be able to judge accordingly. Should the tests continue to be doubtful-positive ( $\pm$ ), the institution of antisyphilitic treatment must be based on a more thorough search of clinical manifestations.

2. *In Untreated Cases of Syphilis.*—We are accustomed to think that a patient suffering from syphilis has *syphilitic antibodies* in his blood and that the Wassermann test proves the presence or absence of these antibodies. This hypothesis renders the interpretation of Wassermann results quite difficult. We know that in most infections, antibodies circulate in the blood long after the disappearance of the infective agent from the body, while in syphilis, just as soon as the *spirochete* are exterminated from the body, the Wassermann test to our knowledge becomes negative. What is probably more likely, is that the Wassermann test is an index to the presence in the system of the *Spirochete pallida* or some product of these organisms, which some writers speak of as syphilitic reagin. And with this view in mind, the interpretation of the Wassermann test in the various stages of syphilis is relatively simple.

In the early primary stage, when the amount of syphilitic reagin circulating in the blood is comparatively small, the Wassermann reaction is likely to be negative or very weakly positive. In the later primary stage as the reagin increases in the system, the Wassermann reaction usually becomes stronger; while in the still later primary stage, still stronger. Craig, who has made a study of the appearance of the Wassermann reaction in 600 cases of primary syphilis, found 36 per cent positive reactions during the first week after the appearance of the chancre; 59 per cent during the second week; 68 per cent during the third week; 77 per cent during the fourth week; and 81 per cent during the fifth week.

It is evident that a negative Wassermann result during the primary stage does not exclude the possibility of syphilis. Furthermore, a one-plus (-) or plus-minus ( $\pm$ ) reaction in this stage might safely be considered as sufficient cause for specific treatment. When the Wassermann is negative in the primary stage, we usually recommend that another blood specimen be forwarded for reexamination in a week or ten days.

In untreated cases of secondary syphilis the Wassermann test is positive in from 92 to 100 per cent of cases. The intensity of the reaction, however, does not always correspond to the severity of the infection, although, as a rule, the more severe the infection, the stronger the Wassermann reaction. It is well to point out in this connection that precocious malignant syphilis, which is perhaps one of the worst manifestations of this disease, gives, as a rule, a negative Wassermann reaction (Wile).

The Wassermann test in tertiary syphilis is positive in about 96 per cent of cases. This test is particularly valuable in this stage of the disease, since the clinical symptoms are, in many cases, obscure. In this condition, Wassermann tests on the spinal fluid are often of great value, particularly when the blood gives a weak or negative reaction.

In latent syphilis where the *spirochete* are dormant and little tissue destruction is going on, one finds a smaller per cent of positives as compared with more



active syphilitic conditions. Craig reports 67 per cent of positive reactions in latent syphilis. Due to the dormant condition of the organisms, one also is likely to find numerous weak reactions in this stage.

In this form of syphilis, particularly in the presence of nervous symptoms, the Wassermann reaction of the spinal fluid is frequently positive when the blood reaction is negative. It is important, therefore, to determine the Wassermann reaction of this fluid in all cases of suspected latent syphilis which give negative reactions in the blood. A positive reaction in the spinal fluid often establishes a diagnosis of neurosyphilis long before nervous manifestations set in.

In congenital syphilis the Wassermann test shows a high per cent of positives. Children showing syphilitic lesions at birth usually give positive Wassermans in 100 per cent of cases. Where the lesions appear later in life, the Wassermann test, it is claimed, misses occasional cases of this form of syphilis.

*It is evident from the foregoing that in every syphilitic stage isolated cases are found which will not respond to the Wassermann test. Syphilologists insist, therefore, that in the presence of clinical symptoms a negative Wassermann test does not exclude syphilis.*

3. *In Cases Undergoing Treatment.*—There is much difference of opinion as to the interpretation of the Wassermann test in treated cases. According to Wile and Hasley,<sup>20</sup> a patient who has undergone intensive treatment and is clinically free from the disease may, with reasonable safety, be considered cured, even though his blood gives a positive Wassermann reaction. "We are convinced," say these investigators, "that in the presence of an intensive therapy, a positive test does not necessarily mean living spirochetes and potential syphilis any more than a positive tuberculin test in an individual who has had tuberculosis would indicate the presence of living tubercle bacilli." This view is shared by numerous syphilologists.<sup>21</sup> There are others,<sup>22</sup> however, who insist that a patient cannot be considered cured unless he gives a negative Wassermann reaction. In summing up this subject, Kolmer<sup>23</sup> states, "It has been abundantly proved, however, that in syphilis a single negative reaction is not sufficient or definite evidence that a cure has been effected, for the disease may recur after treatment is discontinued, at least to the extent that the Wassermann reaction reappears, followed by clinical manifestations. It is necessary, therefore, that successive examinations be made during a period of at least two years, and off and on during the remainder of life." It appears that Wile and his school, particularly object to the so-called "serologic cure," i. e., the employment of the Wassermann test as the criterion as to whether or not a patient is cured. The biology of the Wassermann test being unknown, they insist that the clinical condition of the patient must be of first consideration and the Wassermann results of secondary consideration, in attempting to judge the results of treatment. To these workers, the presence of a positive Wassermann reaction implies that a patient has or has had syphilis.

It would seem that each case represents its own individual problem. And the consensus of opinion among syphilologists appears to be that in the general run of cases, treatment should be continued until the Wassermann is

negative. In the so-called "Wassermann fast" cases, however, where the Wassermann reaction is persistently 4+, the primary aim should be to clear up the clinical symptoms and to institute further treatment only after considerable periods of rest.\*

We are frequently asked how long after the administration of antisyphilitic treatment should one attempt to determine the value of the treatment by means of the Wassermann test. For two or three weeks following treatment, the Wassermann reaction becomes weak or negative in most cases. Frequently, however, this is but a temporary manifestation due to the immediate effect of treatment. If the Wassermann reaction is negative two months or longer after treatment, it is reasonably safe to assume that the treatment had its desired effect. Needless to say that the retesting of blood of supposedly cured cases, let us say, at six-month or yearly intervals should be encouraged in all cases.

4. *The Provocative Wassermann.*—Occasionally the administration of antisyphilitic treatment, such as mercury or salvarsan, will in the course of about three days change the Wassermann reaction of a syphilitic patient from negative to positive. This is known as a *Provocative Wassermann Reaction* and is claimed to occur only in latent cases of syphilis. The explanation of this reaction is not quite clear. The antisyphilitic treatment possibly awakens the dormant spirochete into greater activity, thus causing them to liberate sufficient *reagin* to render the test positive. There is, however, a tendency on the part of syphilologists to place less and less faith in the provocative Wassermann reaction.<sup>24</sup> Some workers believe, for example, that the administration of salvarsan or neosalvarsan will, in some cases, bring about a positive Wassermann reaction even in nonsyphilitic conditions. Strickler, Munson and Sidlick<sup>25</sup> recently reported a series of cases which developed positive Wassermann reactions after administration of salvarsan, although, in their opinion, syphilis could absolutely be excluded from them.

With regard to the spinal fluid, Solomon and Klauder<sup>26</sup> report in a recent paper that the intravenous or intraspinal injection of arsphenamine changed negative to positive Wassermann reactions in a number of syphilitic cases, no provocative reaction being obtained with the blood serum in these cases. These workers, however, do not claim this to be a frequent phenomenon. Vascular neurosyphilis, for example, with negative cerebrospinal fluid frequently does not react in this manner.

The consensus of opinion of syphilologists appears to be that the development of a positive reaction following salvarsan and neosalvarsan treatment should be interpreted with due caution and in conjunction with the clinical evidence of the case.

5. *The Wassermann Test in Pregnancy.*—Prof. J. W. Williams<sup>27</sup> of Johns Hopkins University has recently completed some studies on the significance of the Wassermann test in obstetrics, which throws much light on this highly im-

\*It must be remembered that the designation of 4+ is arbitrary; some syphilitic sera, as determined by special procedure, are as strongly positive as 10+. A persistent 4+, therefore, does not necessarily imply that the antisyphilitic treatment is having no effect on the Wassermann reaction. A syphilitic serum may be 3+ before treatment; 0+ after the first course, and 6+ after the second course of treatment, although the regular laboratory report would be 4+ in each of these examinations.

portant problem. For the past several years, a Wassermann reaction was determined in every pregnant woman who registered in the Dispensary of Johns Hopkins Hospital, and, if it was found to be positive, the patient, whenever possible, was subjected to antisyphilitic treatment. Of 421 women who gave positive Wassermann reactions on admission, 157 did not receive any treatment (group a); 103 received inefficient treatment (group b); and 163 received satisfactory treatment (group c). The result of treatment is shown by the fact that 52 per cent of children of group a were born dead or presented some clinical evidence of syphilis, as compared with 37 per cent in group b and 7 per cent in group c. To quote Professor Williams, "The evidence at our disposal shows that, if syphilis is recognized early in the pregnant woman and is intensively and appropriately treated, almost ideal results may be obtained, so far as the child is concerned."

It occasionally happens that a woman will give a positive Wassermann reaction during pregnancy; give birth to a syphilitic child; and in some weeks after childbirth, give a negative Wassermann reaction. There is no satisfactory explanation for this phenomenon. Neither is there an explanation for the fact that a pregnant woman will in some instances give a negative Wassermann reaction, and yet give birth to a syphilitic child.

6. *A Positive Wassermann in the Absence of Clinical Evidence.*—In the early history of the Wassermann test, numerous workers reported positive reactions in many conditions other than syphilis. In carcinoma, for example, as well as in diabetes, the Wassermann test has been reported positive by various workers. These reports, we believe, were due to the fact that in the hands of early workers the technic of the Wassermann test was not sufficiently fine.

One still occasionally hears that a positive reaction is obtained in scarlet fever. Recent work, however, appears to disprove this. Kolmer, for example, tested the blood of 250 cases of scarlet fever with only 2 per cent of positive results and in this small number syphilis could not be excluded. As pointed out above, one of the important nonsyphilitic diseases which gives a positive Wassermann reaction is frambesia (yaws) which is caused by an organism morphologically similar to the *Spirocheta pallidum* (*Spirocheta pertenuis*). Positive reactions have also been reported in leprosy, in the febrile stage of malaria, in relapsing fever, and occasionally in pellagra. When these conditions are eliminated, a 4-plus reaction should be considered a symptom of syphilis even in the absence of clinical evidence.

In this connection, it may be worth while to give the unanimous report of the Committee on Diagnosis and Treatment of Syphilis of the All American Conference on Venereal Diseases held in Washington in December, 1920, under the auspices of Dr. C. C. Pierce, Ass't Surgeon General, U. S. Public Health Service. (Members present: Drs. Wm. Edler, H. H. Hazen, Wm. A. Pusey, W. Wende, J. S. Salas and A. B. Vasconcelos.)

*Question:* To what extent should a positive Wassermann be relied upon as evidence of syphilis in the absence of clinical symptoms and signs?

*Answer:* That a frank, reliable Wassermann should be regarded as a symptom of syphilis.

*Caution:* (a) In the absence of all other evidences of syphilis, a diagnosis of syphilis upon a positive Wassermann reaction alone should be made with great caution.

(b) In the absence of all other evidences of syphilis, the Wassermann result should be verified by several repetitions at the hands of different observers, if possible. The presence of other conditions which might cause a positive Wassermann should be excluded as far as possible.

(c) There should be a very careful search for other evidences of syphilis, including clinical, serologic, pathologic, and other examinations.

*Conclusion:* After all of the above conditions have been complied with, a positive Wassermann should be assumed to be evidence of the existence of syphilis.

Needless to say that weak-positive reactions should be fully correlated with the clinical signs before reaching a positive diagnosis. The blood of normal individuals will occasionally give a one-plus ( + ) or plus-minus (  $\pm$  ), particularly with a cholesterinized antigen. These weak reactions, in the absence of clinical symptoms or history, could undoubtedly be considered negative.

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## TWO STAINS USED IN PREFERENCE TO WRIGHT'S STAIN IN THE ROUTINE STAINING OF BLOOD SMEARS

BY GARNET B. GRANT, B.S., M.D., AND ERIC R. WILSON, M.A., LOS ANGELES, CAL.

WE have found that the following stains used in the routine staining of blood smears for differential counts are much more satisfactory than Wright's, Jenner's, or any of the other more common stains. Moreover the smear does not require preliminary fixation.

Solution 1. Sat. sol. eosin in methyl alcohol

Solution 2. Mallory's instantaneous hematoxylin

*Technic.*—Smear should be even and not too heavy.

Dry in air or pass through flame.

Stain with solution Sat. sol. methyl eosin, (sol. No. 1) 2 to 2½ minutes.

Wash in distilled water.

Stain with sol. No. 2 hematoxylin 3 to 4 minutes.

Wash in distilled water, blot and dry.

This stain will be found entirely satisfactory for differential counts, but is unsatisfactory for staining malarial parasites, and on this account have adopted the use of the following stain as a routine on all smears suspected of malaria.

Solution 1. Sat. sol. eosin in methyl alcohol

Solution 2. 0.25% aqueous sol. azure 11

*Technic.*—Smear should be even and not too heavy.

Dry in air or pass through flame.

Stain with solution No. 1, 2 to 2½ minutes. (methyl eosin.)

Wash in distilled water.

Stain with sol. No. 2 (Aqueous Azure 11) 20 to 40 seconds

Wash in distilled water—blot—dry.

While this latter makes a most beautiful stain and is well adapted for all routine work including the staining of the malarial parasites, in unskilled hands there will be found a tendency to overstain with the second solution (Azure 11).

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## EDITORIALS

### *Diseases of Animals Communicable to Man*

HOBDAY<sup>1</sup> gives the following list of diseases among animals in Great Britain which are communicable to man: Glanders, anthrax, tuberculosis, rabies, foot and mouth disease, mange of all animals (horse, ox, camel, dog, and cat), certain forms of seborrhea, and pyorrhea. Hobday says that pyorrhea is very prevalent in pet dogs and he thinks it highly possible that it may be transmitted from dogs to men or in reverse order. Of course, certain parasitic diseases in addition to those above mentioned, such as echinococcus, taenia, and trichina, are also transmissible from the lower animals to man; in fact, residence in the two hosts is necessary in order to complete the life cycle of these parasites.

Through the aid of the mallein test and the destruction of glandered animals, glanders in Great Britain has decreased almost to the vanishing point. In 1901, 2,370 horses were destroyed on account of glanders in Great Britain, and 1,828 of these were found in London. The veterinary department of the Ministry of Agriculture shows that during the year 1920 only fifteen outbreaks of this disease occurred in the whole of the British Isles, with a total

destruction of only twenty-two animals, and it is well to remember that these occurred after the sale and distribution of more than 150,000 army horses and mules gathered together from various countries and employed in the war. Twenty years ago it was a daily occurrence for a veterinarian to be called to one of the municipal stables in London to inspect the horses and within one year in one stable of 2,000 horses ninety glandered ones were found. At the present time the use of mallein and its repeated use is compulsory. When an outbreak of this disease is reported in any stable every animal in the stable is quarantined and tested. All ponies sent into the mines and all horses and mules used for special purposes are given the mallein test. During the war special caution was taken to isolate and test all purchased animals. All units were malleined at regular intervals and each individual animal was tested before being sold, even though its destination was the slaughter-house and its flesh was to be served as food for man. Hobday states that in the whole allied forces there was no army whose animals were so free from glanders as the British. It seems quite certain that glanders will soon be unknown in the British Isles.

During the year 1919, 180 outbreaks of infection with anthrax occurred in England and Scotland. It seems that Wales was absolutely free from this disease and had been for three years previously. In all, 275 cattle, eight horses, one sheep, thirty-eight pigs, one dog, and seven ferrets, were affected. Twenty-five per cent of the outbreaks occurred on premises where anthrax had been in evidence during the previous year. The veterinary minister states that the number of cases of anthrax in animals has been reduced from about 1,000 in 1910 to less than 300 in 1919. The sources of infection from anthrax in Great Britain are: (1) Effluents from tanneries passing into streams. (2) Feeding infected offal to pigs. (3) Use of imported foodstuffs and artificial manures. (4) Contaminated sewage. (5) Infected brushes and furs.

For twenty years before the War, there had been no rabies in England. This was due to the close supervision and quarantining of dogs imported into that country. The first case was brought into Plymouth during the month of May, 1918, and during 1919 there were 143 cases of rabies in England among animals, 140 in dogs, two in horses, and one in a pig.

Great Britain was free from foot and mouth disease from 1895 to 1899 and again from 1903 to 1908. During 1920 there was an outbreak of obscure origin. When we remember what great destruction foot and mouth disease has caused in other countries, we can understand the desirability of excluding it. In 1919, northern Italy was visited by a very virulent form of this disease and many of the farmers were ruined in consequence of it.

There are three varieties of parasites that cause mange in domestic animals, and this disease is widely distributed. In the horse, or rather on the horse, the three varieties embrace a sarcopt, a psoropt, and a symbiot, but the chief trouble is due to the two first mentioned and they are transferred to man, especially to individuals engaged in feeding and grooming horses. Mange as transferred from horse to man most frequently infects the surface of the forearm and the hands. Man may become infected by riding mangy horses. During the war this infection frequently occurred, and in these cases

the disease was in evidence on the inside of the legs and in the pubic region. Persons engaged in skinning mangy horses are likely to be infected. Sarcoptic mange is not easily got rid of on horses and requires a treatment which may extend through several months. The psoroptic variety is much more amenable to treatment. In Great Britain mange is a notifiable disease. Sulphur in some form or another forms the basis of most treatments. Hobday says that mange in the dog and cat is transmissible to man, and Whittfield has recently reported seventeen cases in which he confirmed the existence of the sarcoptic parasite in man and traced it to the dog. Owners of mangy dogs often complain of an irritable skin, usually on the inside of the forearm where the dog rests its head while being petted. The warm flesh of the man causes the parasite to leave its canine host and take up its residence on human tissue where it is capable of producing marked irritation. In one of the seventeen cases reported by Whittfield the infected region was on the neck and chest where the owner was in the habit of allowing his pet dog to lie. The lesions are described by Whittfield as small vesicles surrounded by narrow zones of hyperemia, very like that of varicella, but only about one-eighth the size. These lesions are scattered discretely over the surface and are not in groups. If left untreated this parasite may live on man for about six weeks, but under proper sulphur treatment it is easily got rid of. Mangy cats, especially male animals, wander widely and spread their parasites freely.

In the country calves, and in the city cats, are chiefly instrumental in the distribution of ringworm. This infection is often quite resistant to treatment. Hobday complains because there is no adequate meat inspection in England, and consequently measly meat is frequently marketed. Fortunately, the Englishman is not given to eating raw meat and parasites of this nature are generally killed in the process of cooking.

—V. C. V.



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## *ORIGINAL ARTICLES*

### AN ACCURATE METHOD FOR THE CLINICAL DETERMINATION OF EARLY ARTERIAL DISEASE\*

BY HARLOW BROOKS, M.D., NEW YORK CITY

THE diagnosis of arterial disease must be made early if it is to be utilized to any definite practical end in clinical cases. By the older methods, early diagnosis is rarely or never possible except largely on supposition or on historical grounds. As a result very little curative effect is accomplished in cases of arteriosclerosis except in early specific cases in which, as a rule, the diagnosis of arterial disease is based upon the law of probability, rather than on actual detection of changes in the blood vessels.

Thus far early arterial disease has been detected chiefly by an ophthalmoscopic study of the retinal arterioles. This has indeed proved most valuable, but its successful employment presupposes a considerable technical skill with the ophthalmoscope and a very wide experience is necessary for accuracy. The method at its best is hardly applicable for routine use in the hands of the average clinician and yet the data which its utilization furnishes is material of the most elemental importance in the subsequent treatment of any case.

If real results are to be expected in the management of this type of disease, it must be instituted very early and as a rule, this is not possible or probable under usual diagnostic routine, or until such time as irradicable lesions have been inflicted.

It is generally supposed that the retinal arteries are among the very first to show indications of developing arterial disease and this is in all probability a correct statement, but we must recall that in very many cases, especially in arteriosclerosis, lesions are not always diffuse or universal, but are more or less limited to or more pronounced in certain systems or distributions. Even

\*Presented before the Association of American Physicians, Atlantic City, N. J., May 12, 1921.

if this assumption be accepted, it still remains that the retinal vessels correctly typify those of the most primitively important centers, that is, those of the cerebrospinal axis.

The method to which I wish to call your attention was originally demonstrated to me by Dr. David Dennis of Erie, Pennsylvania, who already has made several contributions to medical literature concerning the method, chiefly in ophthalmologic journals.

It is based on the fact that the conjunctival arterioles and other blood vessels show changes quite consonant with those present in the retinae. That this is a justifiable assumption is borne out by the fact that the conjunctival arterioles originate from the anterior and long ciliary branches of the ophthalmic artery and to a lesser extent from the external and internal palpebral branches. They thus represent in a very typical way the circulatory paths within the brain, quite as much so at least as the retinal arteries which spring from the same trunk.

Study of the vessels is accomplished by the use of two very simple and easily manipulated instruments, which are usually in the pocket of the average practitioner. The ordinary pocket electric flash light of which the most convenient for this purpose is the "fountain pen" type is used for illumination. The patient is directed to turn his eyes to either the one side or the other, and the light held at a distance of about three to four cm. is directed obliquely onto the ocular conjunctiva. Study of the vessels is then made through an ordinary ophthalmologist's loupe, which is the most adaptable to the purpose, though other lenses are also fairly satisfactory. The loupe is held at the proper focal distance and for most satisfactory study the eye of the observer is brought close to the lens, just as in the use of the microscope. The vessels under study in the various levels of the membrane are brought sharply into focus by moving the lens to and fro and for the purpose of steadying it the fingers of the lens hand may be rested on the orbital arch of the patient. The study may be made in the diffuse light of the examining rooms or even more satisfactorily in the dark room. When my flash light has been too weak I have secured my lighting by the use of the ordinary head mirror and the usual examining lamp, such as one uses in the examination of the nose and throat. A sharply condensing light, such as is employed in the illumination of the sinuses and so on, is of course very desirable, but the ordinary small, electric flash is all that is needed.

One is thus able to study in a most satisfactory way the larger branches of the ciliaries and palpebrals, the tiny arborizing arterioles and even the capillary circulation in the ocular conjunctivae as well as the return circulation in the venules and the larger ciliary and palpebral branches of the ophthalmic. These vessels are displayed, as it were, beautifully spread out on an ideal pearly white background which forms a most pleasing contrast to the yellow red of the arterioles and the deeper carmine color of the venules. The arrangement for study is as definite and satisfactory as is possible in a well-stained and technically perfect microscopic slide, and by slight variations in focus a vessel may be followed throughout its entire arborization. The method is much more satisfactory than the study of the retinal circulation

because of the sharp contrast of the white background as compared to the pinkish tinge of the retina and it is much more representative of the internal cerebral circulatory condition than the tiny retinal artery since we have here represented for our study four systems of arteries all derived from the same branch of the internal carotid, namely from the ophthalmic. A further great advantage over the study of the retinal artery is represented in that we are here able to observe in the conjunctival circulation the effects of arterial disease on the adjacent tissue. This is particularly fascinating in cases of arterioecapillary fibrosis, in calcifications, fatty degenerations and in instances of hypertension of marked degree. A disadvantage of the method lies in the fact that in cases in which disease of the conjunctiva is present, local vascular lesions are found, particularly in conjunctivitis and in the membrane of patients who have been exposed to the traumatism of wind, intense light, and of irritating chemicals and gases.

The studies of Dr. Dennis of Erie and of Dr. Reese of New York have shown quite finally that these changes in the conjunctival vessels are entirely comparable in any given instance with the alterations manifest in the retinal arteries; this I have also corroborated within the past four years that I have been using the method as a routine, insofar as my skill with the ophthalmoscope may be trusted.

The great advantage to the clinician in the method is that a highly sufficient technical skill may be acquired with a few days' practice. It demands no special instruments and less time is required for the intimate study of the minute circulatory changes in the cerebral vessels than is necessary for a reasonably careful palpation of the radial, brachial or temporal arteries. There is no longer any excuse for mere speculation as to the condition of the blood vessels of the brain, from 30 to 120 seconds of time is sufficient to give one a very satisfactory and entirely accurate knowledge of such changes, if present.

I have found the study most helpful and of tremendous advantage in the detection of the earlier changes present as in syphilis and arteritis from any cause. I have become particularly interested in the study of the terminal arteriole changes of hypertension.

One of the pitfalls into which you will sooner or later fall if you follow this method is the employment of the corneal microscope, by means of which the most intimate study may be made, not only of the microscopic changes in the walls of the arterioles, capillaries and venules, but also the physiologic and pathologic variations in the circulating stream can be made out with greater lucidity than is possible in the web of the frog's foot under low powers of the microscope. This requires, and I can abundantly testify, very considerable degree of technical skill, costly and delicate instruments and most expensive of all, a very large amount of time and patience. This means a study of the conjunctival circulation, however, is indispensable to check up as it were, the gross pathology observable with the hand lens. However, the short distance which I have already traveled into the art shows me that it offers a most valuable method for the microscopic study of the circulatory conditions of the brain. This is transferable by inference to the other parts of the body in gen-

eral arterial disease. It is a study which bids fair to be of even greater value to the internist than to the ophthalmologist.

The most signal benefit conferred by the methods presented is, of course, the ready detection of the very early changes in the arterioles, which may lead on to generalized arterial lesion that plays so important a rôle in the evolution of many internal diseases. By these studies one may detect sufficiently early changes in the arterial system to permit him to inaugurate modifications in habits of life, exercise, diet, etc., also to render possible, in association with proper medication, the introduction of a treatment sufficiently early so that real preventive measures are attainable in many cases.

I have used the study of the conjunctival vessels as a routine in my physical examination of office cases and to a considerable degree also on my hospital services now for about three years. I have been able to satisfy myself that the conjunctival vessels very satisfactorily represent the existence and type of a general arteriosclerosis as confirmed finally by the usual methods of study.

Changes detectable in the conjunctival vessels admit of practically the same classification which we recognize pathologically in our study of anatomical material and a little technical experience readily permits the early recognition of these changes as it were "in vivo."

I wish to present here very briefly a study which I have made of the conjunctival vessels in cases of hypertension. One of the most striking changes which the observer notes in cases of hypertension is the elevation of the more superficial vessels above the surface of the conjunctiva, so that when the light is directed diagonally on the surface of the conjunctiva a reticular framework made up of the tense blood vessels is shown. The arterioles show in early phases also a clear sharp line of demarcation, sharply differentiated off from the surrounding tissues as though overtensely filled. This is manifest even when there are extensive degenerative, infiltratory or inflammatory alterations in the surrounding tissues.

Many of the more superficial vessels are thrown into numerous convolutions as is seen grossly in the contorted arteries and the calcifications of old age. It must be noted, however, that the venules of the conjunctivæ are often markedly convoluted and that this is apparently a normal condition in certain persons, for I have observed its frequent presence in the conjunctival veins of infants and youths entirely independent of any apparent circulatory disease. This is not, however, true of the arteries in which convolutions apparently bespeak only a disease process associated with arteriosclerotic changes or with hypertension.

When a period of hypertension of long standing has been succeeded by a hypotension as from a circulatory decompensation, this elevation and contortion of the arterioles is still preserved and may serve as a sign apparently that a hypertensive condition has preexisted.

Another very frequent, but by no means constant finding in the conjunctival vessels in hypertensive cases is the existence of nodes of lumen contraction so that though the vessel walls are represented by a relatively broad yel-

lowish or whitish streak, but a mere trickle of golden red represents the contracted lumen, at the proximal end of which a minute dilatation of caliber is demonstrable. This is particularly evident in very long standing cases and it apparently represents an arteritis or arteriofibrosis with obliteration of the lumen. The extreme stage of this alteration is represented by complete obliteration so that vessels are represented merely by yellowish or grayish cord-like structures. Where many of these obliterated vessels are present in the conjunctiva, the whole membrane is given a grayish or yellowish white color so that an appearance of extreme anemia may be presented while the blood picture shows a normal condition of affairs quite surprising to the clinician who is accustomed to judge as to the degree of anemia from the color imparted by the conjunctival vessels. This statement comprehends the palpebral as well as the ocular conjunctiva.

The first two signs of hypertension appear relatively early in cases of hypertension, the latter only in the long standing cases.

Tiny aneurysmal dilatations of the conjunctival arterioles are not commonly seen in hypertensive cases. They are, as Dennis has stated to be the case, most common in instances of true arteritis where local changes in or about the arterioles predominate but, of course, they may on theoretical grounds be expected in those cases where an arterio-capillary fibrosis is the cause of the hypertension.

## VENTILATION, WEATHER, AND THE COMMON COLD\*

### A STUDY OF THE PREVALENCE OF RESPIRATORY AFFECTIONS AMONG SCHOOL CHILDREN AND THEIR ASSOCIATION WITH SCHOOL VENTILATION AND THE SEASONAL CHANGES IN WEATHER

BY GEORGE T. PALMER, M.S., EPIDEMIOLOGIST  
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#### INTRODUCTORY

**D**URING the last twenty years there has been a great amount of experimental work on ventilation and its effect on the body. Workers in Europe and the United States are in substantial agreement that it is the thermal factors—temperature, air motion and humidity—which exercise the greatest influence on human comfort, health and efficiency. The chemical composition of the air we ordinarily breathe—leaving out of consideration for the moment those special industrial problems involving gases, fumes and dusts—is of relatively little moment in its effect on human conduct. School children are far better off in a cool, airy room, regardless of the carbon dioxide content of the air, than they are in air virgin pure chemically which is overheated.

It is most important that the facts as we have stated them should be clearly understood, for otherwise, there is bound to arise, as there has in the past, a misunderstanding as to the suitability of different methods of ventilating school buildings.

If variable, as opposed to uniform, temperature, air motion and humidity are desirable factors, then very satisfactory conditions can be maintained in school rooms by ventilating with the windows, protected by deflectors, and an exhaust duct on the opposite side of the room, heating being by direct radiation beneath the windows. This method of ventilating will not always give good aeration. At times the room will be amply flushed with outside air. At other times, due to shifting winds, the circulation will be lessened, the room will not be thoroughly flushed, and the carbon dioxide content will rise, indicating an accumulation of the products of exhalation and body vaporization. Even though the aeration of the room fluctuates, it is possible to have coolness at all times and variability, and if the room is cool and

\*This is an abridged form of a dissertation presented in partial fulfillment of the requirements for the degree of Doctor of Public Health at the University of Michigan, 1920.

This study was conducted jointly by the Bureau of Child Hygiene of the New York City Department of Health, represented by Dr. S. Josephine Baker, Chief of the Bureau, and the New York State Commission on Ventilation, represented by the author, who then held the position of chief of the investigating staff. The collection of sickness records and the taking of temperature and other observations under conditions was done by nurses and physicians of the Health Department under the supervision of Drs. J. Mearns and R. H. Wallis. The routine clerical work of tabulation was likewise conducted by the Health Department under the immediate direction of Dr. Franklin Van Wart. The planning of the investigation, selection of schools, initial instruction of the field staff and the final analysis and interpretation of results is largely the work of the author.

variable within certain limits it makes little practical difference as to the humidity.

On the other hand, if the experimental data of the last two decades are wrong in minimizing the relative value of chemical purity of the atmosphere, then window ventilation as we have described it is inadequate, and it will be necessary to insure at all times voluminous and continuous flushing of the room with outside air. This can be done only by mechanical means, that is by plenum fans or blowers.

There has existed for some time a controversy as to the relative merits of natural and mechanical ventilation. An inheritance from the days of Pettenkofer, when chemical purity was regarded as vital, has kept alive the carbon dioxide content as the standard of ventilation goodness. This standard persists to this day. Measured in these terms the window ventilated room falls into disrepute. A carbon dioxide standard of 6 to 10 parts per 10,000 automatically throws any form of window ventilation into the discard. It is only by mechanical means that this degree of chemical purity can be at all times assured.

With the development of mechanical ventilation there has grown up an impression that uniformity in temperature, in air motion and in humidity is ideal, and again the mechanically forced ventilation far excels the gravity method in this respect. Furthermore the dust in outside air can be removed readily under the mechanical system by the introduction of air washers. This is not possible with window ventilation.

There is much then that can be accomplished with the plenum system that is not possible under the window method. The question arises as to whether the superiority of the mechanical system is superficial, a matter of a relatively unimportant refinement so far as the school classroom is concerned. The drawbacks to the mechanically ventilated classroom are its tendency to overheating, its unstimulating uniformity and its greater expense.

There is much to be said on both sides. The advocates of window ventilation are impressed by its success with tuberculously inclined and undernourished children. It has a wide application in our public schools at the present time. If good for sick children, why not for well children? Is window ventilation in the schoolroom to be ruled out of consideration merely because it fails to live up to the carbon dioxide standard? It was for the purpose of testing out these principles on a practical scale that the present experiment was undertaken. After all, the proof of the pudding is in the eating. If the health of school children, as measured by the amount of respiratory illness, such as colds, tonsillitis, etc., is better under the more elaborate systems of mechanical ventilation, then let us proceed to equip our buildings in this manner. If, on the other hand, the mechanical ventilating equipment does not supply substantial benefits to the health, comfort or efficiency of our school child population, or is actually inferior in the things that count for health, let us face the matter squarely.

There are innumerable demands for public funds. If we are paying out vast sums of money for benefits which are not real but imaginary, recognition

of this fact cannot come too soon. If window ventilation provides the important essentials of a healthy atmosphere in a way that cannot be attained or improved upon by mechanical systems of indirect ventilation, then our school buildings should be built accordingly, and we should not hesitate because a worn out standard of ventilation dictates otherwise.

During the latter part of 1916, from February 14th to April 6th (8 weeks), and the winter of 1916-17, from October 30 to January 26 (12 weeks), observations were made on the health of 5500 New York City school children who were exposed to various types of ventilation in 12 different school buildings. In general these ventilation systems may be classified under three main headings, as follows:

A—Cold, open window rooms, gravity exhaust.

B—Cool, window ventilated rooms, gravity exhaust.

C—Plenum, fan ventilated rooms with gravity exhaust and with windows closed.

As an index of health, the sickness records of the pupils were used. The condition of the air was determined by readings of temperature and humidity and by the personal sensations of the observers as to temperature, moisture, air motion and odor. It would have been desirable to determine also the carbon dioxide content of the air, but this involved analytical work which the staff was unable to do. The absence of these latter data was not serious, however, for the Ventilation Commission had available a mass of data on this subject collected over two years' time, and it was well established that the carbon dioxide content of fan ventilated rooms averages several parts lower than in window ventilated rooms.

For the convenience of the reader we shall reserve the description of working methods and ventilation types in individual schools for the later pages and shall proceed with an account of the findings of this study.

#### RESULTS OF SICKNESS SURVEY IN DIFFERENT TYPES OF VENTILATED SCHOOL ROOMS

The first half of the study in the spring of 1916 covered 2500 pupils in 58 classrooms distributed among 8 schools. The second half in the winter of 1916-17 was represented by 3000 pupils in 76 classrooms in 12 schools.

In both studies the absences due to respiratory illness and the respiratory illness among pupils present in school was greatest in the fan ventilated rooms, Type C. This is the result after combining all records and disregarding in this instance the balancing of the type of pupil, location of school, etc., which will be treated more at length later on.

The excess of respiratory illness in the Type C rooms holds good both for absentees and those in school. The total illness is least in the second group, or cool, window ventilated rooms. The difference, however, between the first and second types of window ventilation is less than between either the first or second and the third. In other words, assuming for the moment that these differences are due to atmospheric influences, the air conditions in the first two types do not produce greatly divergent effects, but the influences at work in the third type are distinctly less favorable.



The significance of these two sets of results may be expressed in this manner; for every 100 cases of respiratory illness in the cool window ventilated rooms, there are 152 in cold, window ventilated rooms, and 231 in fan ventilated rooms.

TABLE I  
RESPIRATORY ILLNESS PER 1000 REGISTRATION  
(PUPIL-SESSION) UNITS\*

Ventilation Type	FIRST STUDY			SECOND STUDY		
	Among Absentees	Among Pupils in School	Total	Among Absentees	Among Pupils in School	Total
A-Cold, Window Rooms	10.6	37.2	47.8	9.2	75.3	84.5
B-Cool, Window Rooms	10.2	22.1	32.3	10.7	44.1	54.8
C-Fan Ventilated	14.2	76.0	90.2	13.0	98.4	111.4

TABLE II  
BASIC FIGURES FROM WHICH RATES IN TABLE I ARE COMPUTED

Ventilation Type	Total Registration Units	FIRST STUDY		Total Registration Units	SECOND STUDY	
		Total Absence Units due to Respiratory Illness	Total Units of Respiratory Illness among Pupils in Attendance		Total Absence Units due to Respiratory Illness	Total Units of Respiratory Illness among Pupils in Attendance
A	61,658	655	2,298	89,067	822	6,705
B	71,231	728	1,578	113,959	1,218	4,961
C	65,088	925	4,950	115,215	1,497	11,329

The actual temperature conditions found in the three types of rooms are disclosed in the two tables following, in one of which results are expressed as averages, and in the other by temperature groups.

TABLE III  
AVERAGE OF ROOM TEMPERATURES

Ventilation Type	First Study	Second Study	Mean
A	58.8	59.1	59.0
B	66.9	65.9	66.4
C	68.8	67.9	68.4

\*The unit of illness was one pupil per half-day school session. Illness is reported in pupil session units. One pupil ill ten sessions counted the same as ten pupils ill one session. A pupil was continued on the register regardless of the length of absence from school unless it was found that the family had moved away from the school district, or that the pupil had left school permanently.

Separate tabulations have been made of illness resulting in absence from school and illness among pupils who continued to attend school. The absences are classified as (1) absence from respiratory illness, including coryza, bronchitis, pharyngitis, laryngitis, tonsillitis, pneumonia, tuberculosis and a miscellaneous group variously termed gripe, colds, so-called throat, etc.; (2) absence from illness other than respiratory, namely: stomachache, backache, broken leg, etc. In this group were also the acute infectious diseases such as diphtheria, measles, scarlet fever, whooping cough, chickenpox, mumps, etc. This was done because it was felt that the spread of these infections was largely determined by specific susceptibility rather than by atmospheric influences; (3) absence due to causes other than illness, such as staying at home to mind the baby, going on a visit, shopping with parents, truancy, etc.

The fan ventilated or Type C rooms averaged but two degrees higher than those of Type B and nearly ten degrees warmer than Type A. Type A was extremely cold for a school room. Type B was cooler than is customarily found. The average temperature of Type C, though higher than B, was not exceptionally high.

There was a wide range in the daily temperatures, which are lost sight of in the averages.

TABLE IV  
FREQUENCY DISTRIBUTION OF TEMPERATURES  
PER CENT OF SESSIONS

Types Ventilation	59° and below	60-69°	70° and over
	<i>1st Study</i>		
A	56	41	3
B	5	61	34
C	4	57	39
	<i>2nd Study</i>		
A	46	48	6
B	8	88	4
C	0.2	99	0.8

The A rooms rarely reached 70°. Fully half of the sessions were below 59°. The B and C rooms rarely fell below 60°. The temperatures in the second study were much more uniform, particularly as regards Type C and to a lesser extent Type B. More than one-third of the sessions in B and C in the first study were above 70°. Much of this represented an overheated condition. However, the first study was made in the late winter, when greater outdoor variation is experienced than from November to January. Although the proportion of sessions between 60 and 70° does not appear to differ much in the B and C rooms, yet there was an appreciable difference within this range. Thus, in the first study 24 per cent of sessions in B were from 60° to 64°, whereas but 6 per cent of the sessions in C were within this range. Thirty-seven per cent of B sessions were from 65 to 69 as against 51 per cent of the C sessions. The C rooms were warmer, as the averages have already indicated.

The interesting point to be noted is that whereas there was but two degrees difference in temperature between the B and C rooms, there was a wide difference in the sickness rates. Between 7 and 8 degrees separated Types A and B, and yet in spite of this the sickness rates were quite similar. Evidently some factor other than temperature operated differently on these three classes of rooms or else a rise of two degrees above 66° is far more conducive to colds than a drop of as much as 7 degrees.

#### HUMIDITY

The relative humidity ranged from 38 to 16% and did not differ greatly in the three classes.

As would be expected, the relative humidity was higher in the colder rooms. With the same amount of moisture present as in Type A, the relative humidity in the first study at the temperature in Type B would be 33.4.

TABLE V  
RELATIVE HUMIDITY

Ventilation Type	First Study	Second Study
A	43.7	46.3
B	37.8	43.0
C	37.8	41.2

and in Type C 31.4. The corresponding figures in the second study would be 29.9 for B and 28.7 for C. It would appear, therefore, that there was an accumulation of moisture in the Type B rooms, and this could only come from reduced aeration. In Type C the same explanation would hold. The rooms at P. S. 59 were humidified, as were also the rooms at P. S. 51 and 97, but this would hardly affect the average of all rooms in the group to this extent. It is also possible that the taking of the humidity reading was not as accurately done in the dry rooms, the wet bulb being read before the mercury column had completed its fall. These readings are higher than we should expect at this season of the year, judging from records taken by the Commission in similar rooms in other schools.

Other indices of air conditions were the opinions of the nurses who visited the rooms at least twice daily.

## FRESHNESS AND ODOR

The results of the nurses' votes on the freshness or lack of freshness and presence of odor in the rooms are given in Table VI.

TABLE VI

Ventilation Type	PERCENTAGE OF SESSIONS JUDGED		Odorous
	Exceptionally Fresh	Odor Absent but not Exceptionally fresh	
	<i>First Study</i>		
A	62	24	14
B	25	57	18
C	21	64	15
	<i>Second Study</i>		
A	69	28	3.2
B	18	62	20
C	22	67	11

The results are very interesting from several points of view. The Type B rooms are most odorous in both studies. The excess over the others was slight in the first study, 18, as compared to 15 for Type C and 14 for A. It was more marked in the second study, 20, as against 11 for C and only 3.2 for A.

The freshest rooms are the coldest rooms. Sixty-two per cent of the sessions in Type A in the first study were judged exceptionally fresh, and in the second study the figure was 69 per cent. The figures for Type B were 25 per cent in the first and 18 in the second. Type C had 21 per cent in the first and 22 per cent in the second.

What seems clear from these figures is that to be exceptionally fresh the greater part of the time, rooms must be well below 65 degrees in temperature. Warmer rooms may be free from odor and yet exceptionally fresh not much over one-fifth of the time.

In rooms that did not differ greatly in temperature, as B and C, the greater aeration produced by fan ventilation reduced odor to a slight degree in the first study, and to a marked degree in the second. It failed to make the rooms any fresher in the first study, but did help out in the second study.

Although we do not possess complete records of the carbon dioxide content, there is little doubt but that the smallest amount would be found in the fan ventilated rooms, Type C, as we have already pointed out. Repeated records collected in three schools show this tendency (Table VII).

TABLE VII  
CARBON DIOXIDE IN PARTS PER 10,000

SCHOOL	VENTILATION	
	B	C
33	6.9	5.5
115	7.6	6.5
97	8.6	5.7

The markedly greater freshness of the Type A rooms is due, without question, to their low temperature, and not because of the greater chemical purity of the air. Freshness is not a question of odor, for the Type B rooms were fresher than C and yet more odorous.

#### SENSATION OF TEMPERATURE

The recorded votes of the nurses as to whether the temperature of the rooms felt "too cool," "satisfactory" or "too warm" are given in Table VIII.

TABLE VIII

VENTILATION TYPE	PER CENT OF SESSIONS JUDGED		
	TOO COOL	SATISFACTORY	TOO WARM
<i>First Study</i>			
A	7.9	77	16
B	6.2	78	16
C	10	76	14
<i>Second Study</i>			
A	26	70	3.9
B	8.6	80	11
C	3.6	85	12

In the first study the per cent of satisfactory sessions was about the same in all three types, being in the neighborhood of 77 per cent. There were more sessions judged "too warm" in Types A and B than in C, although the average temperature was lower than C. The "too cool" sessions were most numerous in Type C, the rooms of highest temperature. In other words, the coldest rooms felt warmer than the warm rooms. In view of the actual temperature found, it would appear that the nurses were influenced in their judgment of what the

temperature should have been rather than by actual sensation. In no other way can we account for the votes in rooms whose temperature differed by at least ten degrees, as was the case in Types A and C.

The votes in the second study more nearly reveal the actual temperature condition as indicated by the thermometer. In A 26 per cent of the sessions were judged "too cool." In fact, 4 per cent were voted "too cold"—an extreme condition. In Type B, 8.6 per cent were "too cool" and in C 3.6 per cent.

Type C had the greatest number of sessions judged satisfactory as to temperature; namely, 85 per cent. The corresponding figure for Type B was 80 and for A, 70.

Too great warmth was experienced 12 per cent of the time in C, 11 per cent in B and but 3.9 per cent in A.

The sensation of temperature reflects the actual thermometer reading—to some extent at least. There was much less overheating in the second study, and this agrees with the lesser number of "too warm" votes.

#### SENSATION OF MOISTURE

As will be seen from the figures given below, over 80 per cent of sessions in all three types of the first study were judged satisfactory as to moisture. Both moisture and dryness were most pronounced in Type C.

TABLE IX

VENTILATION TYPE	PER CENT OF SESSIONS JUDGED		DRY
	MOIST	SATISFACTORY	
	<i>First Study</i>		
A	5.9	88	6.7
B	1.7	89	9.0
C	7.3	83	9.7
	<i>Second Study</i>		
A	33	66	6.6
B	20	74	6.4
C	8.5	84	7.9

The second study reveals a greater divergence between the rooms. Type C had the highest percentage of satisfactory sessions, 84. Type B had but 74 per cent and Type A, only 66. The cooler sessions are associated with moisture, the warmer sessions with dryness. One-third of all sessions in A were moist and less than 1 per cent dry. Twenty per cent of sessions in B were moist and 6.4 per cent dry. Eight and five-tenths per cent of C were moist and 7.9 per cent dry.

There was less overheating in the second study and the sensation of dryness is less. Type C is similar in both studies. The other two types are different in that the second study shows many more moist sessions.

#### AIR MOTION

The greatest proportion of satisfactory votes as to air motion was found in A. Moving air was noticed most frequently in A and least in B. The results

of both studies are similar, although it is surprising to find the sessions in Type C judged "dead" to be more numerous in the second study where the air flow through the rooms was greater and the temperature was lower.

TABLE X  
PER CENT OF SESSIONS JUDGED

VENTILATION TYPE	DEAD	SATISFACTORY	BREEZY
<i>First Study</i>			
A	4.7	81	14
B	15	78	7.1
C	9.3	73	15
<i>Second Study</i>			
A	9.2	74	17
B	18	75	7.0
C	23	68	9.8

(To be continued.)

# A REVIEW OF NINETY-FOUR NECROPSIES. WITH SPECIAL REFERENCE TO THE PNEUMONIAS\*

BY GEO. W. COVEY, A.M., M.D., LINCOLN, NEBR.

## INTRODUCTION

UNFORTUNATELY the pathologic service in our center overseas was undermanned and underequipped, and the work had to be done under very harassing conditions. In a period of less than five months more than 30,000 patients passed through this center, of whom 429 died. Of these, 80.3 per cent were autopsied—a total of 356 necropsies. The greater part of these were done by my chief, Dr. Moses Barron, pathologist of the University of Minnesota, and by myself. More than one-half of the deaths occurred in one month—October, 1918, the daily average being eight. In addition, Dr. Barron was chief of the laboratory service, and I examined more than a thousand tissues microscopically during this time. Of the 106 postmortems done by me, I have complete records of the gross findings in 94.

A very complete and cleverly written résumé of the 356 cases has been published in the *Archives of Internal Medicine* for September, 1919, by Dr. Barron. In this he has touched on most of the important pathologic lesions that came under our observation. My excuse, therefore, for a paper on my own records is to make a somewhat more detailed study of a few points concerning the heart, aorta, kidneys, and sinuses, and to dwell especially on the pneumonias which we encountered.

## HEART

One of the most striking features of our heart findings was the paucity of chronic lesions. In other words, the heart boards had efficiently ruled out chronic diseases in this field.

I have here recorded the weight of each of 61 hearts (Table I). The min-

TABLE I

Number of hearts—weights recorded	61
Average weight heart: males—Morris Grey	341 grams
Average weight hearts in this series	331.7 "
Maximum weight	450 "
Minimum weight	220 "
Number weighing more than the average	29 "

imum was 220 grams, the maximum 450 grams. Eleven hearts weighed 400 grams or more. Twenty-nine weighed more than 340 grams. The average

\*Read before the Lancaster County Medical Society, April, 1920.

weight was 331.7 grams. The average weight of hearts in the male recorded by Morris and by Grey is 341 grams. We see, therefore, that our general average falls about 10 grams low, but that 29, or 47.5 per cent, were above the average.

Seven hearts, or 7.4 per cent, showed a patent foramen ovale (Table II).

TABLE II

Patent foramen ovale	7 or 7.4 per cent
Number hearts with valvular lesion	11 or 10.3 " "
Number hearts with acute valvular endocarditis	4 or 3.7 " "
Number hearts with acute endocarditis, mitral alone	1 or 0.9 " "
Number hearts with acute endocarditis, mitral and tricuspid	1 or 0.9 " "
Number hearts with acute endocarditis, mitral and chronic elsewhere	2 or 1.8 " "
Number hearts with acute endocarditis, aortic	0

Eleven of the 94 hearts, or 10.3 per cent, showed valvular lesions. Only four of these were acute infections. The chronic lesions were universally of a mild and almost insignificant degree. The mitral valve was affected in five cases (4.7 per cent), four of which were acute. Only one of these was an acute lesion of the mitral valve alone; one occurred with an acute endocarditis of the tricuspid valve, and two with chronic disease of the aortic valve. No acute lesions were seen affecting the aortic valve, but it had some slight chronic affection in eight cases.

The myocardium showed some evidence of degeneration, such as a brownish color and flabby consistency in eight cases, or 7.5 per cent. None of these was very marked, and no acute infectious process was seen here. Dilatation is recorded in nine cases, or 14.7 per cent.

Twenty-five cases, or 23.5 per cent, showed some lesion of the pericardium or epicardium. Five of these were acute, three being a real acute pericarditis and two subpericardial hemorrhages. The remainder consisted of some remnant of past disease of the heart coverings, the most interesting of which were the so-called "soldier-spots." A true soldier-spot is a thickened area of epicardium corresponding to a complementary "spot" on the pericardium, varying from a few millimeters to  $1\frac{1}{2}$  cm. or more in size. Some were found in which a fibrous band, running from epicardium to pericardium, connected these two "spots." In others the band had apparently gone further, the fibrous brush of tissue being absorbed and leaving a coin-like whitish thickening on the two membranes. Six of these cases, or 5.64 per cent, showed typical "soldier-spots," while 13 others showed smaller or larger areas of thickened epicardium alone.

#### AORTA

Five aortas had evidence of aortitis, nonluetie in character, marked enough to be considered abnormal; yet none of them was remarkable in extent or degree. Three cases of true syphilitic aortitis, each with aneurysm, were posted in the Center, and all of them happened to fall in my own service. Only one of these was suspected prior to death. This was a large sacular aneurysm beginning at



the aortic ring and involving practically the ascending limb of the arch. Death occurred here due to sudden dilatation of the aortic ring. The two others were typically luetic throughout, but the aneurysm occurred in the abdominal portion. In one of these, death was due to rupture and infiltration of the retro-peritoneal tissues with blood. An interesting angle of this case is the fact that a tumor of the right kidney was diagnosed. The abdomen was opened and explored by the eye and finger of the surgeon, who decided the tumor was too extensive for removal and contented himself with abducting the man's appendix. Even then it remained for the pathologist to show that the tumor behind the right kidney was blood from the aneurysmal sac. The third was entirely unsuspected and was found in a man who died of septicemia. It was a sac lying between the aorta and the vertebrae in the lumbar region. It had eroded a portion of the bodies of two vertebrae, but had caused no symptoms.

KIDNEY (SEE TABLE III)

In 69 cases the average weight of the left kidney is 186 grams; that of the right is 184. The minimum weight is 120 grams and the maximum 300 grams.

TABLE III

Number kidneys weighed—pairs	69
Average weight kidneys in male—Kelly and Burnham	168 grams
Minimum weight kidneys in male—Kelly and Burnham	107 "
Maximum weight kidneys in male—Kelly and Burnham	264 "
Average weight left kidney, this series	186 "
Average weight right kidney, this series	184 "
Minimum weight kidney, this series	120 "
Maximum weight kidney, this series	300 "
Congenital abnormalities	2 or 1.8 percent
Acute nephritis	28 or 26.3 " "
Chronic nephritis	14 or 13.2 " "
Nephrolithiasis	2 or 1.8 " "
Acute pyelitis and hydronephrosis	1 or .9 " "
Pyelonephritis	1 or .9 " "
Cystic degeneration	1 or .9 " "

Two cases had but one kidney each, one being a horseshoe kidney weighing 500 grams and having two pelves and two ureters, the other being a single right kidney weighing 300 grams with aplasia of the left kidney and ureter. According to Kelly and Burnham (*Diseases of the Kidneys, Ureters and Bladder*, Vol. I, p. 137) the normal weight of a kidney in the state of physiologic distention is 168 grams—average; the minimum 107 and maximum 264 grams. The right usually weighs slightly less than the left.

In 28 cases the kidneys showed acute nephritis; however, most of these were mild, only two being severe and the cause of death. In 14 cases mild chronic nephritis was present.

In addition, calculi were found in two cases; septic infarcts with abscess formation in two cases; acute pyelitis in two cases—one having an acute pyelitis on one side with a hydronephrosis on the opposite side; pyelonephritis in one case, and cystic degeneration in one case.

## SINUSES OF SKULL, MASTOIDS AND MIDDLE EAR (SEE TABLE IV)

TABLE IV

Purulent sinusitis	37 or 34.8	per cent
Single sinus or pair	31 or 29.3	" "
More than one pair	6 or 5.6	" "
Pan sinusitis	2 or 1.8	" "
Sphenoidals	34	
Frontals	5	
Ethmoidals	4	
Middle ear	7	
Mastoid	2	

Purulent sinusitis was present in 37 cases, or 34.8 per cent, a single sinus or pair being infected in 31 cases and more than one pair in six cases. All the sinuses were infected in each of two cases. The sphenoidals were involved 34 times, the frontals 5 times, the ethmoidals 4 times, the middle ear 7 times, and the mastoids 2 times.

## THE PNEUMONIAS



Fig. 1. Photomicrograph from a section of lung showing bronchopneumonia. Shows a bronchiole filled with pus. The contiguous alveoli only are filled with exudate. 1. Lumen of bronchiole. 2. Wall of bronchiole. 3. Alveoli filled with exudate.

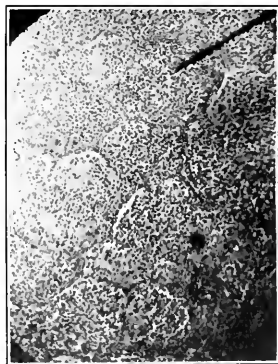


Fig. 2.—Section of typical lobar pneumonia. Note thin alveolar walls and abundance of exudate in the alveoli.

Most (about 60 per cent) of the cases showing sinus involvement were pneumonias, and a large percentage occurred during the month of October, 1918, together with the first days of November. The organisms found in the sinuses were usually identical with those from the lung or blood stream.

Of the 94 cases under discussion, 57, or 60.6 per cent, showed pneumonia. Of these, 50, or 87.7 per cent, were of the bronchogenic type; two, or 1.8 per cent, were lobar and bronchial; five, or 9 per cent, were classified as interstitial.

The most common complications of these were pleuritis and sinusitis. There

was fibrinous or serofibrinous pleurisy in 22, empyema in 10, and sinusitis, either single or multiple or pan sinusitis, in 21.

I have records of the bacteria found in 32 cases. The pneumococcus was found 14 times, the streptococcus 18 times, *B. Welchii* twice, and *B. pyocyaneus* once. In several cases more than one organism was present.

Influenza was the initial diagnosis in nine, or 16.2 per cent, of these cases.

Seventy-five per cent of the bronchopneumonias were bilateral, many being so massive that practically whole lobes or complete lungs were solid.

Among these cases there was none of pure ordinary lobar pneumonia.

I do not need to discuss the pathology of the ordinary lobar pneumonia except to call to your minds for comparison the principal points. Bronchopneumonia (Fig. 1), as you remember, has its origin in the finer radicles of the bronchi and spreads outward into the surrounding parenchyma. The "lobu-



Fig. 3.—Photomicrograph of section of lung showing marked interstitial pneumonia. Note the extreme thickness and cellularity of the alveolar walls and the scarcity of exudate in the alveoli.

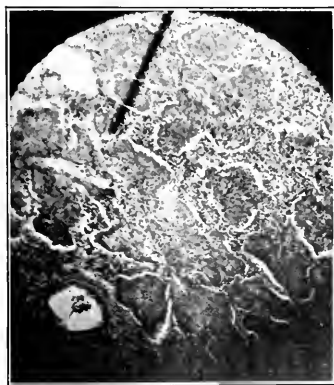


Fig. 4.—Photomicrograph of section of lung showing interstitial pneumonia. This is very similar to Fig. 3 except that the alveoli are filled with fibrinous exudate containing very few cells.

lar" idea is slightly misleading. In sections from various parts of these infected lungs we can trace the different steps in the pathologic process. Bronchioles can be seen containing exudate and having more or less degeneration and desquamation of the cells of the mucous membrane, with congestion and dilatation of the capillaries but no surrounding inflammation. Others can be found about which perhaps but one or at most a few concentric layers of alveoli contain exudate (Fig. 1). From this we pass to bronchioles having a more or less definite concentric area of consolidation involving a narrower or wider area of parenchyma. This area is always surrounded by a zone of atelectatic alveoli, due to pressure of the distended consolidated portion, and this in turn by an area of emphysematous alveoli—probably an effort on the part of the body to compensate for the functional loss of lung tissue. These areas spread ever outward until they may meet, become confluent, and give a massive consolidation.

The old text book picture of mononuclear cells with no fibrin in the bronchopneumonias and polymorphonuclear cells, with much fibrin in the lobar pneumonias, must be largely overdrawn, especially as regards the fibrin deposit, for in our studies we found many bronchogenic pneumonias having as great or greater amounts of fibrin than the lobars; this more particularly in cases having been gassed, but also in others.

In lobar pneumonia (Fig. 2) the greater part or the whole of one lobe or many lobes becomes involved at once. There is congestion followed by the pouring out into the alveolar spaces of an exudate rich in red cells. In a few hours (or, at most, days) these become broken up and are gradually replaced by white cells, largely of the polymorphonuclear type. Lysis of the exudate begins, the bronchioles then become flooded with pus, the cells of which are rapidly undergoing degeneration, and the lung returns gradually to its normal state with

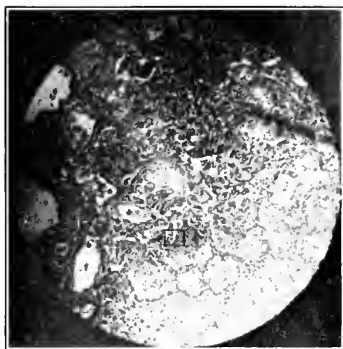


Fig. 5. Photomicrograph of section of lung showing interstitial pneumonia. Here organization has begun. Exudate contains many fibroblasts originating in alveolar walls. Many alveoli collapsed.



Fig. 6. Photomicrograph oil immersion of portion of section in Fig. 5 contained in the square. 1. Exudate in alveolus. 2. Wall of alveolus. 3. Fibroblasts apparently originating in wall.

nothing to denote that it has been infected. We have then the stages of congestion, red hepatization, gray hepatization, resolution, and recovery.

Early in our work we encountered a relatively large number of unusual pneumonias which at first we were at a loss to name. The involvement was either lobar or bronchial. The area was airless and firm, but had a very different feel than usual. It was not quite so firm as a lobar, and not friable, but of a rubbery resiliency, it being difficult to press the finger into it hard enough to tear it, and on being released from pressure it immediately returned to its normal contour. The pleural surface was often covered by fibrinous exudate. It was deep red in color, and there were frequently subpleural pinpoint hemorrhages. The cut surface is dark red in color or may have brownish areas and occasionally small flecks of yellow. The surface is somewhat moist, but on pressure a very little fluid, not of a purulent character, is expressed. When scraped dry, a universal fine, lace appearance results.

When these lungs were studied microscopically we found the reason for the gross appearance (Figs. 3-6). We also found that other pneumonias, bronchial or lobar, frequently showed similar small scattered areas, and that occasionally we were deceived in the gross specimens by other lesions, such as edema of the alveolar walls. Microscopically, the striking feature of these sections was a relatively immense thickening of the alveolar walls, with very little exudate in the alveoli themselves (Fig. 3). This it is that gives the characteristic gross appearance described above. In the more acute types there is more alveolar exudate than in the more chronic. This consists of serofibrinous material with scattered cells, a few of which are polymorphonuclear leucocytes, the remainder mononuclear cells, lymphocytes and desquamated endothelium (Fig. 5).

The alveolar walls are edematous and their capillaries are dilated and injected with red cells, but the striking feature is their marked cellular infiltration (Fig. 3). They are often crowded full of cells, at times polymorphonuclear, with scattered large mononuclear cells; again, almost entirely with the mononuclear variety.

In a given section areas may be seen where the walls are thickened and the alveoli are filled with exudate as described. Other areas will show no exudate (Fig. 5). In the less acute forms, organization is beginning to take place. Areas are seen where the exudate has disappeared from the alveoli and they have collapsed, permitting the thick walls to come in contact and making a strand of solid tissue with here and there the suggestion of an alveolar space. Other portions show fibroblastic invasion of alveolar exudate, the new cells originating in the walls (Figs. 5 and 6). Still others give the appearance of new connective tissue, with here and there a space either without endothelium or with a partial lining of cuboidal or columnar cells produced by the attempt at regeneration.

## LABORATORY METHODS

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### A METHOD FOR THE DETERMINATION OF BLOOD VOLUME<sup>2\*</sup>

BY ELIZABETH FRANKE AND STANLEY R. BENEDICT, NEW YORK CITY

METHODS available for the determination of blood volume depend upon one of three general procedures. The direct method, devised by Haller, Wrisberg<sup>1</sup> and Weleker,<sup>2</sup> and modified by Plesch<sup>3</sup> and others<sup>4</sup> depends on direct estimation of the total hemoglobin content of the body. This procedure is probably the most accurate, but is applicable only where the life of the experimental animal is sacrificed, and the technic involved is laborious. The second group of methods, developed by Gréhand and Quinaud,<sup>5</sup> Haldane,<sup>6</sup> Van Slyke<sup>7</sup> and others,<sup>8</sup> depends upon inhalation of a gas (carbon monoxide) which will combine with a constituent of the blood (hemoglobin) so that by subsequent determination of the new compound formed, or of the concentration of the gas in the blood, the blood volume may be calculated. This type of method has not met wide approval on account of the unsatisfactory technic involved, and because the results obtained show a wide variance from those given by other methods.

The third, and most widely used group of methods, depends upon the introduction of a known quantity of a substance into the circulating blood with subsequent determination of the concentration of the substance in a sample of blood withdrawn from the circulation. Such a procedure must depend upon the use of a substance for injection which remains practically unaltered in the blood stream for sufficient length of time to permit thorough mixing with the entire blood, and for withdrawal of the sample for analysis.

Acacia, employed by Meek and Gasser,<sup>9</sup> conforms to these conditions but the complicated analytical technic required to determine acacia with accuracy has limited the general usefulness of the method. An adaptation of this method, using the refractive index of the nonprotein fraction of the serum has been proposed by McQuarrie and Davis. This procedure is applicable to small quantities of blood and is probably quite accurate when a satisfactory refractometer is available, and conditions are properly controlled.

Theoretically, perhaps the simplest method of blood volume determination would depend on the introduction into the circulation of a nondiffusible non-toxic substance having an intense color, so that the concentration of the substance would be determined colorimetrically directly in a portion of plasma. Keith, Rowntree and Geraghty first proposed a method based upon this procedure and employed "vital red" a German dye, as the substance for injection. Vital red does not penetrate the corpuscles and leaves the circulation compara-

\*From the Department of Chemistry, Cornell University Medical College, New York City, and the W. A. Clark Special Research Fund.

tively slowly. Difficulty in obtaining satisfactory samples of the dye in recent years has limited the availability of the method for general use. The work of Harris, Hooper, Smith, Belt and Whipple, and Dawson, Evans and Whipple has indicated that the colorimetric principle for blood volume determination as introduced by Rowntree and collaborators is the most generally satisfactory and simple procedure, provided one secures a dye which can be relied upon not to penetrate corpuscles or tissues within a reasonable period of time.

On account of the difficulties at present in obtaining reliable dye preparations which do not penetrate cells or tissues it occurred to us that hemoglobin might be used with advantage in the determination of blood volume. Hemoglobin is nontoxic, and owing to its high molecular weight and intense coloring power should be admirably adapted for use where a nondiffusible colored product is desired. Our experiments have shown that oxyhemoglobin does not penetrate the red cells to the slightest degree, and remains in the circulation without detectable alteration for periods of time quite sufficient to allow of thorough mixing with the blood and removal of samples for analysis. The final concentration of hemoglobin in the plasma is readily determined by comparison in a colorimeter with a portion of the solution injected, after suitable dilution. It is unnecessary to use purified hemoglobin for the determination. Results are exactly as satisfactory where a solution obtained by laking corpuscles with water, followed by addition of enough salt to make the whole solution isotonic, is employed. The hemoglobin employed need not come from the same species of animal as the one used in the experiment. We have used beef hemoglobin in our dog experiment with exactly the same results as where dog hemoglobin was employed. Injection can be made as rapidly as possible without detectable effect on the general condition of the animal.

#### DESCRIPTION OF THE METHOD

The principle of the method is the introduction into the blood stream of a solution of hemoglobin, prepared by laking blood, and the subsequent determination colorimetrically of the concentration of this hemoglobin in the plasma by comparison with a suitable standard mixture of hemoglobin and plasma. The volume of the corpuscles in the blood must also be determined. This can be done either with a hematocrit, or by the method described below, which depends upon addition of hemoglobin solution to whole blood, and the subsequent determination of the hemoglobin concentration in a portion of the plasma. The three main steps in the procedure are:

1. Withdrawal of a preliminary sample of blood to secure plasma for use in the standard solution, and to furnish a sample of blood for determination of the corpuscle volume.
2. Injection of hemoglobin solution.
3. Withdrawal of blood samples a few minutes later in which the concentration of hemoglobin in the plasma is determined colorimetrically.

#### PREPARATION OF HEMOGLOBIN SOLUTION FOR INJECTION

Centrifuge a known amount of blood (a few c.c. more than 0.5 c.c. for each kilogram of body weight of the subject) in a sterile centrifuge tube until

the corpuscles have settled fairly completely. Pipette off the plasma as completely as possible into a small graduated cylinder. Add to the cell residue in the centrifuge tube enough sterile water to make the total volume twice that of the original blood. Shake to thoroughly hemolyze the cells. Add 8.5 milligrams of NaCl for each c.c. of water added, and mix by shaking. Centrifuge to remove white cells and debris, and pipette off the clear red solution. Approximately 1 c.c. of this solution is injected for each kilogram of body weight of the subject.

*Technic.*—Take a preliminary sample of blood in the usual way, using about 2 milligrams of potassium oxalate per c.c. to prevent clotting. This sample should have a volume of from 10 to 15 c.c. A portion of this blood (2 c.c.) is measured into a centrifuge tube for the corpuscle volume determination, or a small quantity is used for the hematocrit determination of corpuscle volume. The main portion of this blood is now centrifuged, and while this is going on the previously prepared hemoglobin solution (prepared as described above) is injected intravenously. The quantity used should be about 1 c.c. per kilogram of body weight, and must be accurately measured. Approximately 2 minutes after making the injection a sample of about 10 c.c. of blood should be withdrawn (either from an artery or vein) and if convenient a second sample should be withdrawn about two minutes later for a check determination. These samples of blood should be drawn into vessels containing 2 milligrams of oxalate for each c.c. of blood, and should be centrifuged as promptly as possible after thorough mixing with the oxalate.

#### PREPARATION OF THE STANDARD SOLUTION

The dilution of hemoglobin best adapted for colorimetric determination was found to be approximately that obtained by diluting one part of whole blood to two hundred parts with water. Since the color of plasma varies in different individuals, it is necessary to mix the standard with plasma in approximately the same quantity as is present in the unknown solution. Hence the standard solution is prepared by mixing proper proportions of the hemoglobin solution used for the injection with plasma obtained from the preliminary blood sample, and proper dilution of the mixture with normal salt solution. For preparing the standard solution, dilute one part of the hemoglobin used for injection to five volumes with 0.85 per cent salt, and one part of this solution again to ten volumes with the salt solution. Finally mix a portion of this solution with an equal volume of plasma obtained from the preliminary blood sample. This final solution is employed as standard solution in the colorimeter. For comparison, the hemoglobin-tinged plasma obtained by centrifuging the sample (or samples) withdrawn after the injection is diluted with an equal volume of salt solution. The standard is conveniently set at a height of 15 m.m.

#### CALCULATION OF RESULTS

Let  $S$  represent the height of the standard in millimeters, and  $Y$  represent the reading of the unknown in millimeters. Then  $S/Y^2$  (since the plasma withdrawn was diluted 1:1 with saline) will represent the relative concentra-



tion of hemoglobin in the original plasma as compared with the standard, which figure we will term  $C$ . Then if  $H$  represents the number of c.c. of the hemoglobin solution injected,  $H/C \times 100$  will give the number of c.c. of plasma in the body. The number of c.c. of plasma in the body, divided by the percentage of plasma in the blood, and multiplied by 100 will give the number of c.c. of total blood in the body.

Example: Standard is set at 15 mm., unknown reads 18 mm.  $15/18 \times 2$  gives 1.66. Then if 12 c.c. of the hemoglobin solution were injected  $12/1.66 \times 100$  gives 734, the number of c.c. of plasma in the body. If the blood was 65 per cent plasma then  $734/65 \times 100 = 1129$  c.c. of blood in the body.

#### DETERMINATION OF PLASMA PERCENTAGE IN BLOOD

*Principle.*—The method depends upon mixing hemoglobin with whole blood and determination of the concentration of hemoglobin in the plasma after centrifuging. It offers no special advantage over the use of the hematocrit but is quite accurate and may prove serviceable where a satisfactory hematocrit is not available.

*Procedure.*—To 2 c.c. of the preliminary sample of blood in a centrifuge tube add an equal volume of hemoglobin solution which represents a dilution of whole blood 1:10.13 (hemoglobin solution II). The mixture is centrifuged. One c.c. of the supernatant fluid is diluted to 10 c.c. with 0.85 per cent salt solution and this colored solution read in a colorimeter against a standard solution prepared by treatment of 0.5 of a portion of hemoglobin solution II

TABLE I  
BLOOD VOLUME FIGURES FOR NORMAL DOGS

DOG	WEIGHT	PER CENT CELLS	PER CENT PLASMA	PLASMA VOLUME	BLOOD VOLUME	C.C. BLOOD PER 100 G. BODY WT.	BLOOD VOL. EXPRESSED IN FRACTION OF BODY WT.
20	13.46	32.0	68	725.3	1066.6	7.92	1/12.6
18	8.8	24.0	76	516.7	679.8	7.72	1/12.9
15	16.42	37.4	62.6	842.6	1346.1	8.19	1/12.19
14	10.68	37.0	63.0	530.0	841.3	7.87	1/12.6
22	11.3	37.4	62.6	700.08	1118.3	9.01	1/11.0
12	8.8	36.1	63.9	466.6	730.4	8.30	1/12.0
10	8.8	36.1	63.9	538.2	842.2	9.57	1/10.4
81	14.72	31.6	68.4	823.2	1205.6	8.10	1/12.2
81	14.80	41.3	58.7	676.8	1182.9	7.99	1/12.5
85	11.0	32.0	68.0	715.7	1078.5	9.80	1/10.19
85	10.86	28.0	72.0	744.4	1068.8	9.84	1/10.16
86	16.04	29.2	70.8	1133.3	1620.7	10.1	1/9.89
87	6.4	45.0	55.0	374.8	700.4	10.9	1/9.13
87	5.92	36.0	64.0	342.9	565.7	9.55	1/10.4
90	9.08	37.4	62.6	546.9	894.9	9.85	1/10.4
91	10.60	40.0	60.0	550.38	952.3	8.98	1/11.1
91	10.18	37.4	62.6	548.3	905.8	8.8	1/11.3
92	10.52	45.2	54.8	556.07	1059.7	10.0	1/9.9
93	15.94	40.0	60.0	653.96	1124.9	7.05	1/14.1

with an equal volume of clear plasma from the preliminary blood sample and dilution of the mixture to 10 c.c. with 0.85 per cent salt solution.

*Calculation.*—The figure obtained by dividing the reading of the standard by the reading of the unknown solution gives the relative concentration of hemoglobin in the solution. By dividing the total volume originally in the centrifuge tube by this figure we obtain the actual volume of total fluid present (plasma + hemoglobin solution added). By subtracting the volume of hemoglobin solution added we obtain the volume of plasma. Dividing this by the volume of whole blood and multiplying by 100 gives us the percentage of plasma in the blood.

*Example.*—Standard set at 15 mm.; unknown reads 12.2 mm. Then  $15 \div 12.2 = 1.22$ . 4 c.c. (total volume in centrifuge tube) divided by 1.22 gives 3.278. Subtracting the 2 c.c. of hemoglobin solution added we get the figure 1.278 c.c. as the plasma present in 2 c.c. of blood. The  $1.278 \div 2 \times 100 = 63.9$  per cent of plasma in the blood.

#### CONTROLS OF THE METHOD

*Hemolysis.*—In this method, as in any which depends upon colorimetric determination directly upon the plasma, are disturbing factors. If care is exercised in drawing the blood, so that absolutely no clotting occurs, there will be little trouble from hemolysis. Very prompt centrifugation of the blood samples is also desirable, since blood is apt to hemolyze on standing, even for short periods.

In Table I the results are given of 19 experiments to determine the blood volume of dogs by the method described in this paper. In each case the hemoglobin solutions were injected into the femoral vein and the blood withdrawn through an oiled cannula inserted into the femoral artery on the same side.

TABLE II  
VALUES FOUND IN REPEATED DETERMINATIONS

DOG	TIME INTERVAL	WT. IN KILOG.	PER CENT PLASMA	PLASMA VOLUME	BLOOD VOLUME	C.C. BLOOD PER 100 G. BODY WT.	BLOOD VOL. EXPRESSED IN FRACTION OF BODY WT.
81	11 days	14.72	68.4	823.2	1205.6	8.10	1/12.2
		14.80	58.7	676.8	1182.9	7.99	1/12.5
85	11 days	11.0	68.0	745.7	1078.5	9.80	1/10.19
		10.86	72.0	744.4	1068.7	9.86	1/10.16
91	7 days	10.6	60.0	550.38	952.3	8.98	1/11.1
		10.18	62.6	548.3	905.8	8.8	1/11.2
87	7 days	6.4	55.0	374.8	700.4	10.9	1/9.13
		5.92	64.6	342.9	565.7	9.55	1/10.4

Local cocaine anesthesia was employed. It will be noted that the results vary from 7.7 c.c. to 10.9 c.c. per 100 grams of body weight, or from about 1/10 to 1/14 of the body weight. These results are in agreement with those reported by other observers with other methods.

Table II records duplicate determinations of the blood volume on four

dogs at intervals of seven or eleven days. The results show excellent agreement.

Studies were also made to determine the rate of disappearance of hemoglobin from the circulation. In these experiments samples of blood were taken from a cannula inserted into the femoral artery, at intervals of 1.5, 3, 5, 10 and (in one case) 20 minutes after the hemoglobin injection. The results are summarized in Table III. It will be seen that three minutes after the injection of the hemoglobin more than 98 per cent is still present in the blood stream. Indeed samples taken at 1.5 and at 3.0 minute intervals almost always show excellent agreement. At the end of five minutes the loss of hemoglobin averages about three per cent, and after ten minutes, 4.2 per cent.

Experiments have been carried out to test the accuracy of the method suggested for the determination of plasma and corpuscle volume both by comparison with hematocrit readings and by artificially increasing the corpuscle content of blood samples by removal of measured quantities of plasma. The maximal errors found by the latter method amounted to 2.6 per cent, so that we may conclude that the method is accurate to within two or three per cent.

*Summary.*—A simple method for the determination of plasma percentage and plasma volume in living animals is described. From these two factors the total blood volume can be calculated. The blood volume of normal dogs averages about 1 II of the body weight. While the method has not yet been tried with other animals than dogs, there is no apparent reason that it cannot be applied to any species.

TABLE III  
RELATIVE CONCENTRATION OF HEMOGLOBIN IN PLASMA AFTER ITS INJECTION

DOG	(1.5 minute sample taken as 100)				
	1.5 MIN.	3 MIN.	5 MIN.	10 MIN.	20 MIN.
81	100	96.2	96.2		
81	100	99.2		97.7	86.4
85	100	97.6	97.6	98.1	
85	100	95.9	94.9	93.6	
86	100		93.3	93.3	
87	100	95.5	95.0	90.0	
87	100	102.0	102.0	100.6	
90		100		96.9	
90	100	100.5	98.6	96.5	
91		100	99.1	94	
91	100	101.0	99.7	98.5	
92	100		99.3		
93	100	96.0			

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<sup>18</sup>This principle was employed by Stewart, (*Jour. Physiol.*, 1879, xxiv, 356) who added a solution of oxyhemoglobin to a known volume of centrifuged blood from which a known amount of plasma had been removed, and calculated the plasma volume from the dilution necessary to bring the standard solution to the same tint as that of the unknown (compared in hematinometers).  
<sup>19</sup>This solution can be prepared by dilutions of a portion of hemoglobin solution used for injection with four volumes of 0.85 per cent salt solution, or whole blood may be diluted to ten times its volume with distilled water and the resulting solution treated with 85 milligrams of salt for each c.c. of water added.

## A STANDARD METHOD FOR PREPARING AND STANDARDIZING LIPOIDAL ANTIGENS FOR THE WASSERMANN TEST\*

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SINCE the time when the complement-fixation test has found its practical application as the Wassermann reaction in the diagnosis of syphilis, the study of the nature of lipoidal antigens has deservedly attracted much attention.

Wassermann and Bruck in their original investigations used first watery and later alcoholic extractives of syphilitic livers with the idea that they were dealing with a specific complement-fixation test. This idea, however, was soon abandoned when the nonspecific nature of the syphilitic antigens was known from actual tests conducted with alcoholic extractives of normal tissue rich in lipins. (Marie and Levaditi, Landsteiner, Porges, and Meier Weil and Braun, Noguichi, etc.)

On further study of such tissue extractives (Sachs, Browning and Cruikshank, Walker and Swift) introduced the addition of very small amounts of cholesterol, thus increasing considerably the sensitiveness of plain extracts. Noguichi observed that cholesterol, as well as certain other undesirable products of the nature of proteins soluble in alcohol (that are usually found in ordinary tissue extractives), rendered the antigens less specific in direct proportion to their quantity. Consequently Noguichi devised a method by which he could separate the relatively pure lipins from the other undesirable extractives. In general his method consists in precipitating lipoids from an ethereal solution by the addition of acetone.

As a natural outcome of the above studies we now possess four different

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antigens. The syphilitic tissue extractive, the normal tissue extractive, the cholesterolized or reenforced tissue extractive, and the acetone-insoluble fraction of Noguchi.

A casual examination of the literature on the Wassermann reaction will disclose the fact that the study of the antigen (the most important of all items entering into the reaction) has so far been rather neglected. Much time and labor have been spent in finding some new method to produce a more potent or sensitive antigen, but relatively little has been done to establish a strictly scientific method for the standardization of the complement-fixing value of Wassermann antigens. Thus, for example, all the pioneers in this field of research, Wassermann, Bruck, Sachs, Brownning and McKenzie, Thomas and Ivy, Walker and Swift, Field, M. Stern, etc., still use either an arbitrarily fixed dose of some preparation, or a known fraction of the dose which by itself is not anticomplementary. There is no doubt that almost all of them make a preliminary titration of the relative fixing value of their antigen to determine the specific and nonspecific (anticomplementary) limits; but the way such titrations are made is invariably open to criticism of being far from scientific.

To illustrate the above statement we will briefly explain the method of titration used by the New York Department of Health Laboratories. They take for example as their dose a fixed amount of a given strength of dilution, one-fourth of which should give complete fixation of the unit complement used in the actual test, in presence of a strongly positive serum and which in quantities 3 to 4 times as large should not be anticomplementary.

Before I proceed any further, let me state in advance that given an antigen, irrespective of the method of preparation, the degree of specificity will bear a constant ratio to the amount of lipins present, provided the nonlipin fraction in it is always constant.

This fact, long recognized by workers in this field, such as Field, has led to the attempt of standardizing antigens through determining the amount of solids found in a definite volume of the given antigen. Unfortunately the relative proportion of the lipin and nonlipin fraction in an antigen is not always the same. Field<sup>1</sup> uses an alcoholic extract of guinea pig hearts reenforced with cholesterolin to half saturation. The total solids in his antigens vary from 5 to 8 milligrams per c.c. This variable item is corrected in the final dilution of the antigen, so that one c.c. of this dilution will always represent 0.1 mg. of solids. Noguchi, on the other hand, goes a step further in getting rid of the nonlipin fraction by precipitating it with acetone, thus obtaining the relatively pure semisolid lipins in a weighable form. This method would be the most promising of all the rest were it not for the fact that the acetone-insoluble fraction of tissue extractives does not represent their total antigenic value. On running parallel tests with both fractions, acetone-insoluble and acetone-soluble one can easily demonstrate the fact that acetone-soluble fraction retains a great part of the antigenic element, though it is less specific. It is partly due to this fact that as antigens, comparatively higher concentrations have been used of the acetone-insoluble fraction.

The following will be an attempt to handle the question of antigens in mathematical terms. The basis of all complement-fixation reactions is the degree of complement deviation in terms of hemolysis (the indicator). Consequently the standardization of the hemolytic system precedes that of the antigen. In spite of so many variations introduced in the original Wassermann technique, the standard unit of 1 c.e. of 5 per cent suspension of sheep cells has been more or less adhered to.

Wassermann in his original work used whole blood to make the 5 per cent suspension. This was later replaced by washed, packed cells, thus eliminating another source of error, *i. e.* the variability of the corpuscular volume in whole blood.

Disregarding the possibility of nonuniformity in the resistance of cells of different sheep, let us temporarily adopt a standard unit of cells to which our antigen standardization has to be adjusted. The original unit of 1 c.e. of 5 per cent suspension is inapplicable as a standard unit for two reasons: It demands unnecessary waste of material, especially the complement, and what is far more important, its undue bulk renders it less sensitive as an indicator.

During the last decade the general tendency among workers in this field has been to reduce the Wassermann unit into  $\frac{1}{2}$ ,  $\frac{1}{4}$ , or even  $\frac{1}{5}$  of the original. To safeguard against all these variations let us adopt as our unit 1 c.e. of 1 per cent suspension, as this amount, in addition to increased sensitiveness and economy in material, has the advantage of being easily convertible into the original 5 per cent or any of its fractional units.

*Then our unit complement would be the least amount that is capable of hemolyzing completely 1 c.e. of 1 per cent suspension of properly sensitized cells.*

There is much divergence of opinion regarding the proper sensitization of cells. From 2 up to 10 units of sensitizer have been used by different authors. Fortunately in our antigen titrations we depend on the combined action of complement and sensitized cells. In this way any excess or deficiency in any single item is finally corrected by the other in the titration of the complement prior to antigen titrations.

In our titrations we have invariably adopted the method of titrating the complement against 2 units of sensitizer, which we found very satisfactory. The antigen unit would then be *the least amount capable of deviating completely one unit of complement in presence of exactly one unit of specific reagent*. The significance of this has been only casually dwelt upon. The present system of pluses is a qualitative denotation after all. No two 4-plus sera have necessarily the same amount of specific reagent. Therefore, any attempt to fix a standard for the antigen without at the same time determining the unit specific value of the serum against which the antigen in question is to be titrated is bound to fail. A standard unit of specific reagent, then, is *the least amount capable of deviating one unit of complement in the presence of one unit of antigen*. Having thus defined our terms, we now proceed to the actual application of our method.

## TECHNIC OF THE DETERMINATION OF THE STANDARD UNIT OF ANTIGEN

The whole process is divided into three steps: (1) the determination of the minimal fixing value in presence of a known strong positive serum. (2) the determination of the approximate standard unit of the antigen. (3) the determination of the exact antigenic and specific units.

The process of determining the minimal fixing value of the antigen does not differ much from the technic actually carried in any other laboratory: Make an arbitrary dilution of the antigen, arranged in a series of 5 to 10 test-tubes. Each tube gets one unit of complement.

The unit of the complement is determined in the usual way by finding the minimal amount capable of hemolyzing completely 1 c.c. of 1 per cent sensitized sheep cells. The preparation of 1 per cent sheep erythrocytes needs no special comment, as long as it is serum-free and actually measured. As far as the sensitizer is concerned, two units of sensitizer per unit of cells is safe enough, although a half and half proportion would be more scientific.

Then we add an arbitrary amount of known strong positive serum, say 0.1 c.c. and finally the antigen is added in gradually decreasing doses, say from 1 c.c. to 0.05 c.c. All tubes are now incubated at 37° C. for 30 minutes and 1 c.c. of 1 per cent sensitized sheep cells are added into each tube, incubated another 30 to 45 minutes and the final readings taken. The smallest amount of antigen that gives complete fixation of one unit of complement as used in the tests is considered the minimal fixing value of the antigen in question.

It is customary in different serologic laboratories to use an excess of complement, from 2 to 5 complement units. We adopted the use of 1 such unit for the reason that by doing so, besides the economy in material, we also eliminated a variable factor, and our results can easily be converted at any time in terms of any other laboratory findings.

Having thus determined the minimal fixing value of an antigen, we now proceed to determine the approximate standard unit antigenic value. Suppose the minimal fixing value of the antigen to be 0.5 c.c. of 1:100 dilution. We now make 1:50 dilution (twice as concentrated), and arrange 6 rows of 6 tubes each. (More tubes can later be added if necessary.) Every tube gets the same amount (0.1 c.c.) of the known strong positive syphilitic serum. Next, the complement is added in regularly increasing units, so that the first tube of each row gets 1 unit, the second 2 units, etc., the last tube of each row getting 10 units. Finally we add the antigen in regularly decreasing doses so that all tubes of the first row get 0.05 c.c. each, those of the second row 0.10 c.c. each, etc., tubes of the last row getting 0.50 c.c. each. The deficiency in total volume is made up by adding saline. Then tubes are shaken and incubated for 30 minutes at 37° C., when one unit of sensitized cells is added to each tube. Again they are shaken and further incubated for 30 to 45 minutes at the same temperature, whereupon readings are taken and results tabulated. Table number I will make this clear.

TABLE I

ANTIGEN IN C.C.	COMPLEMENT IN UNITS					
	1	2	3	4	5	10
0.05	c	c	c	c	c	c
0.10	p	c	c	c	c	c
0.20	n	p	c	c	c	c
0.30	n	n	p	c	c	c
0.40	n	n	n	p	c	c
0.50	n	n	n	n	p	c

Explanatory note: c denotes complete hemolysis, p varying degrees of partial hemolysis, and n no hemolysis at all.

## CONCLUSIONS

Table I shows that the approximate standard unit of the antigen in question lies between 0.10 and 0.20 c.c. of the 1 to 50 dilution, probably at 0.15 c.c. But we do not know yet to what degree the antigen unit thus determined is influenced by the strength of the specific reagen. The table also gives an approximate estimation of the extent of this influence, because we at least know that this value is not below 5 units in the standard quantity of 0.10 c.c. above used, since by our definition, 0.10 c.c. of the serum is capable of fixing at least 4 units of complement. Its failure to fix more than 4 units of complement may be due to two reasons: either the serum has no more than 4 units of specific reagen, or if it has the insufficiency of the antigen added renders the serum ineffective as far as fixation is concerned. This aspect of the question will be taken up in the final stage, where the exact unit values of antigen and reagen are definitely fixed. Here we use only one unit of complement for all tubes, varying our antigen and specific reagen within their approximate unit limits.

TABLE II

SERUM IN C.C.	ANTIGEN 1:50 DIL.			
	0.05	0.10	0.15	0.20
0.005	p	p	p	p
0.010	p	p	p	n
0.015	p	p	n	n
0.020	p	p	n	n

Explanatory note: p denotes varying degrees of partial hemolysis, n denotes complete inhibition of hemolysis.

## FINAL CONCLUSION

Table number II shows that 0.005 c.c. of the serum fails to exhibit a unit of reagen, while 0.01 c.c. of serum with 0.20 c.c. of antigen fulfill the established requirements for a unit. 0.015 c.c. of serum with 0.15 c.c. of antigen also fulfill the requirements. Either one of the combinations might be safely used as a standard unit. In our experience we always found it safer to favor the antigen at the expense of the serum, consequently we would adopt as standard unit 0.01 c.c. for the serum and 0.20 c.c. for the antigen in a dilution of 1:50.

The fallacy of standardizing the antigen against any given strong positive serum lies in the fact that all positive sera are not quantitatively alike.

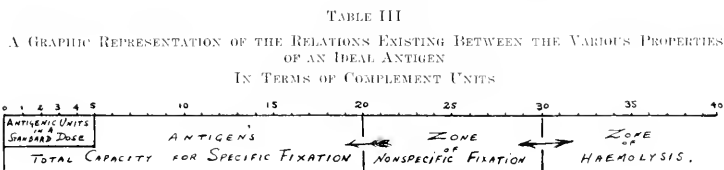


Every laboratory, through habit or for certain special reasons, has sanctioned a definite dose for the specific serum. This dose varies from one drop to 0.20 c.c. The other ingredients vary more or less in proportion to the serum used. If now a serum in the fixed dose of say 0.10 c.c. results in complete fixation of the complement used, it is called a 4-plus serum. By further reducing the amount of serum a limit soon is reached, where any further reduction of the amount will fail to fix the complement completely. Supposing this lowest quantity to be 0.01 c.c., the original dose of 0.10 c.c. must then contain 10 absolute units of specific reagen. By standardizing an antigen against such a strong positive serum we ignore the law of multiple proportions as exhibited by the classic studies of the Bordet and Ehrlich schools; (V. Dungern,<sup>2</sup> Morgenroth and Sachs,<sup>3</sup> Manwaring,<sup>4</sup> etc.).\*

Assuming that we have made clear the question of an absolute unit-fixing value of the antigen we now proceed to determine its specific (non-anticomplementary) and hemolytic values before we decide about its adaptability in the Wassermann reaction. These two values are arrived at as usual by preparing comparatively more concentrated dilutions of the antigen, so that the total volume used in the previous tests is not exceeded.

It is more or less generally agreed upon that with a good Wassermann antigen the minimal anticomplementary value should at least exceed 4 or 5 times the minimal fixing value, and that the more the anticomplementary value exceeds the specific unit, the better and safer the antigen is considered.

The hemolytic element in the antigen, is not often met with. However, occasionally a tissue extractive will exhibit hemolytic properties in amounts very dangerously small, thus interfering with its specific values. Such extractives should be discarded as useless. A hypothetical antigen answering the above description can be represented graphically as follows:



We, therefore, collected antigens from various laboratories in New York City and determined carefully, in accordance with the scheme already outlined, the specific and nonspecific antigenic units. We then computed the ratios of these two values, which for the sake of convenience we called the Specific Coefficient. For example, supposing the unit antigenic value of the given dilution to be 0.10 c.c. and the beginning anticomplementary value of the same dilution at 0.40 c.c., the specific coefficient then would be 0.10/0.40

\*If we could only abolish the unscientific method of pluses and record our results in terms of the lowest complement-fixing values of the specific serum, as it is already done with spinal fluids in most laboratories, we would establish a relatively accurate quantitative method, influenced by only one variable item, the antigen, whose scientific standardization we have undertaken to discuss in the present article.

$= 1_4$ , which means that the antigenic unit is  $1_4$  of the beginning anticomplementary unit.

The antigens under investigation were then grouped, according to their specific coefficients under the following classes: In all 15 antigens were thus examined:

Class 1. All antigens with Specific Coefficient of 1 or above 1. Such antigens are very unsafe, and should be discarded. Out of 15 antigens 2 came under this class, one plain alcoholic and one cholesterinized.

Class 2. Antigens with a Specific Coefficient of  $1_2$ . Of these we had four, two plain alcoholic, one cholesterinized, and one acetone-insoluble fraction.

Class 3. Antigens with a Specific Coefficient  $1_4$ . Two alcoholic and two cholesterinized antigens.

Class 4. Antigens with a Specific Coefficient of  $1_5$ . Under this class we had four; one plain alcoholic, one acetone-insoluble fraction, and two cholesterinized.

Class 5. Antigens with a Specific Coefficient of  $1_{20}$ . Of this class we had only one, a cholesterinized antigen.

Excepting one cholesterinized antigen, with a specific coefficient of  $1_{20}$ , the Specific Coefficient of all the rest varied between  $1_2$  and  $1_5$ ; in other words, the total fixing capacity of these antigens did not exceed 5 units.

Noguchi, in his book on Serum Diagnosis of Syphilis, puts down the total specific fixing value of his antigen as 5 units. Kolmer, referring to the Noguchi antigen, makes the statement that it contains a fixing capacity of 10 to 20 units. Thiele and Embleton<sup>5</sup> use 0.20 c.c. of 1:10 dilution of acetone-insoluble lipins, which they state to be from 50 to 100 units in complement-fixing value. Bronfenbrenner,<sup>6</sup> in his article "A Procedure for the Serum Diagnosis of Syphilis", says, "It is possible to prepare this antigen (acetone insoluble fraction) in such a way that  $1_{10}$  of the anti-complementary dose of it contains from 10 to 100 antigenic units, depending upon the serum against which it is tested."

As far as Noguchi's own statement is concerned, it conforms with ours. Kolmer's figures also must undoubtedly be correct, the higher values he gets being due in all probability to the strong positive sera against which his antigens are titrated. Otherwise, as we have already stated above, were the antigens in question titrated against a single, or at the most, a double unit of specific reagent, the results would not have been very different. Coming to Bronfenbrenner's statement, granted that the samples of acetone-insoluble fractions in our possession were inferior to his, we still fail to understand the wide limit of variation, even assuming that he standardized his antigens against varying degrees of positive sera, considering that an antigen has been used, whose mode of preparation is more standard than of any other Wassermann antigen. Further it must be clearly understood that the unit value of 10 to 100 is contained as stated by Bronfenbrenner, in  $1_{10}$ , the anticomplementary dose, consequently, the total fixing value would be from 100 to 1000 units.

Although Bronfenbrenner does not state the method by which he determines the specific fixing capacity of his antigen, his statement that the

antigenic value depends upon the serum against which it is tested, leads us to infer that he arrived at this conclusion by making quantitative Wassermann tests on different grades of positive sera. According to the Noguchi method of determining quantitatively the specific reagent, the standard amount of patient's serum is reduced to the lowest possible limit that is capable of still fixing one unit of complement. For example if a serum fixes one unit of complement in  $\frac{1}{50}$  or  $\frac{1}{100}$  the standard dose ordinarily used for qualitative determinations it is considered as 50 or 100 unit serum. Thus it is self-evident that, although the quantitative fixing value of the serum used is 50 or 100, the antigen, in the process of determining this value is titrated against a fraction of this value and only *one unit of complement*.

The discussion of the subject of specific coefficient would be incomplete without bringing forth a very important fact concerning the relationship of the specific coefficient with the actual specificity of the antigens under consideration. It must be clearly borne in mind that the specific coefficient gives the relative, not the absolute specific value of an antigen. An antigen with a relatively high specific coefficient, say  $\frac{1}{5}$ , might fail to detect a weak positive serum which another one with a lower specific coefficient,  $\frac{1}{2}$ , might detect. After all, the specific coefficients were determined on very strong positive sera that could be detected by any antigen conforming with the ordinary antigenic requirements, while most of the discrepancies in Wassermann reactions arise from doubtful or weak positive sera.

We dealt with this very interesting question by running several hundred routine Wassermann tests, simultaneously with all antigens in our possession.\* The results are the following:

Cholesterinized antigens give more positives than crude extracts do, with the difference that not all positives with cholesterin antigens are syphilitic. The crude alcoholic extracts detect from 40 to 60 per cent of the sera that are positive with the cholesterin antigens, with the difference, however, that false positives are rare exceptions. Our figures with plain alcoholic antigens are lower than those given by other investigators, especially when they used the ice-box fixation method.

The degree of absolute specificity bears a distinct relation to the specific coefficient. This, however, is not an unexpected finding when we consider the significance of the specific coefficient. An antigen with a total fixing value of 20 units should be superior to one with only 4 units, as the former can be used in amounts as small as  $\frac{1}{10}$  the anticomplementary dose and still represent 2 units while the latter would represent the same value in  $\frac{1}{2}$  the dose, thus rendering its use rather unsafe.

On further investigation we found that the amount of solids contained in a definite volume of antigen, excluding cholesterin and alcohol-soluble

\*Our Wassermann technic consists in using inactivated serum in amounts of 0.05 c.c. for cholesterinized and 0.05 and 0.10 c.c. for plain alcoholic antigens. The complement, guinea pig's serum, is titrated daily against 2 to 4 units of antiserum sensitizer, 0.50 c.c. of 1 per cent sheep cells being used as indicator. For the acid tests 2 such complement units are used. Of cholesterin antigens 2 units and of plain alcoholic extracts 2 to 4 units are used. The antigenic unit determinations were made as described. The total volume in each tube is always 1 c.c. A primary incubation of 30 to 45 minutes is done in a water bath at body temperature for the fixation of the complement. At the end of this time 0.50 c.c. of 1 per cent sensitized sheep cells are added. In about 30 to 45 minutes, when the antigen, complement, and serum controls show 100 per cent hemolysis, the readings are taken.

proteins, bears a direct ratio to the degree of specificity of the antigen in question. The major part of these solids is made up of lipins and certain other bodies akin to it, the discussion of which we will take up later.

The action of cholesterol in Wassermann antigens is still a debated question. There is no doubt that cholesterol increases considerably the sensitiveness of plain extractives, but this is always at the expense of their specificity. In trained hands it is a valuable aid to detect weak positive sera, especially in cases under treatment; but its results must always be checked up with a reliable plain extractive. On the other hand cholesterolized antigens in our experience were almost worthless where active sera were used.

The statement that alcohol is a general protein coagulant is of relative truth only, depending primarily on the relative purity of the alcohol used. For by extracting dry muscle tissue with varying grades of alcohol, from 50 per cent to absolute, we noticed that the more dilute the alcohol, the more protein it will hold in solution. Again the more concentrated it is, the richer it will be in nonprotein extractives.

By far the most interesting of these findings was the peculiar relationship between an antigen's total of solid contents and its specificity. Table Number IV shows very clearly all these relations in the case of a few antigens, taken at random and showing all extreme possibilities.

TABLE IV  
THE SPECIFIC COEFFICIENTS OF VARIOUS ANTIGENS IN RELATION TO THE TOTAL SOLID CONTENTS OF SAME

KINDS OF ANTIGENS USED		TOTAL SOLIDS IN GMS. PER C.C.	ANTIGENIC UNITS IN C.C.	MINIMAL ANTI- COMPLEMENTARY VALUES IN C.C.	SPECIFIC COEFFICIENT	STAND. ANTI- GENIC DOSE IN C.C.	ANTIGENIC UNITS IN STANDARD DOSE
No. 1	(Plain alcoholic extr.)	0.002	0.50	1.00	1 <sub>2</sub>	0.10	0.20
No. 2	(Cholesterolized No. 1)	0.006	0.10	0.25	1 <sub>2.5</sub>	0.10	1.00
No. 3	(Plain alcoholic extr.)	0.012	0.50	1.00	1 <sub>2</sub>	0.50	1.00
No. 4	(Cholesterolized No. 3)	0.016	0.025	0.50	1 <sub>20</sub>	0.10	4.00
No. 5	(Aceton. Insol. Lipins.)	0.030	0.10	0.20	1 <sub>2</sub>	0.10	1.00
No. 6	(Noguchi Antigen)	0.030	0.01	0.20	1 <sub>5</sub>	1.10	2.50
No. 7	(Plain alcoholic extr.)	0.026	0.005	1.00	1 <sub>200</sub>	0.25	50.00
No. 8	(Cholesterolized No. 7)	0.030	0.05	2.50	1 <sub>50</sub>	0.50	10.00

Explanatory notes: Antigen No. 5 is prepared in accordance with the directions of Noguchi: 0.30 gms. of the lipin mass plus 1 c.c. of ether, plus 9 c.c. of methyl alcohol, thus representing 0.03 gms. of solids per 1 c.c. The figures given are from values obtained by titrating it against inactivated specific sera.

Figures in antigen No. 6 are taken direct from Noguchi's titrations as given in his book. Antigen No. 7 was prepared in accordance with the technique described in the latter part of the present article.

Antigen No. 8 is the cholesterolized form of antigen No. 7. While the former shows 50 units per standard dose, it detects only 50 to 60 per cent of those sera that are positive with antigen No. 8.

These findings led us to attempt to prepare an antigen free, as far as possible, of all elements of error and specific in action. On account of the peculiar relationship between an antigen's total of solid contents and its relative specificity, and given that the highest yield in solids is obtained by

keeping the strength of alcohol as high as possible, we devised a technic, whereby it was possible to keep the alcohol at the original strength throughout the whole process of extraction. Thus we were not only eliminating the possibility of diluting the original strength of the alcohol used; but also avoiding the presence of those undesirable water-soluble by-products, which are protein in nature. Noguchi calls them collectively the "proteotropic substances" as they fix complement when mixed with certain proteins such as nucleoproteins, peptone, albumoses and other autolytic decomposition products of protein.

#### TECHNIC OF PREPARING ANTIGEN

Heart muscle tissue (ox, guinea pig, or human) is carefully freed of its connective tissue and fat, washed thoroughly under running water for an hour and gently squeezed from time to time to free it of blood. It is then wiped in cheese cloth, cut into small cubes and chopped finely in a meat chopping machine. Both the liquid and the solid parts are collected and the latter transferred into a meat pressing machine. The liquid that drains out is collected into an evaporating glass jar to which the fluid portion collected during the chopping process is added and there evaporated at body temperature or under the sun to a dry powder. Likewise the solid parts as they come out of the pressing machine are made into thin, flat cakes and dried immediately. The time for drying should not exceed a few hours as otherwise protein disintegration due to autolytic and saprophytic changes might vitiate the results.

The dry tissue is now passed through a nut-grinding machine and then pulverized in a mortar. To this the powder residue of the liquid portion is also added.

To 10 parts of the powder by weight 90 parts of absolute ethyl alcohol of the purest quality obtainable are added, then shaken well and put away in the incubator for extraction. The bottle is shaken once or twice daily. At the end of the week the supernatant amber-yellow clear fluid is decanted carefully into a tightly stoppered glass bottle and stored away in the ice box.

An antigen prepared in this way is very rich in extractives. The chemical composition of these extractives is very complex. The major part is made up of various aminophosphatids. The rest consists of fats, fatty acids, especially stearic and just a faint trace of cholesterol and soluble proteins.

In the course of a few months, during the time the antigen is kept in cold storage, certain chemical changes take place. The fat and fatty acid fractions combine with the alkaline elements present in the extract, thus forming neutral salts, soaps, which being insoluble in alcohol, are precipitated in the form of white chalky granular masses. Part of the fats is also precipitated as such, being caught among the granules of the soaps. Thus the antigen gets seasoned automatically.

An example of such an antigen is antigen No. 7 of the last table. Compared with the other antigens in the same table it shows 0.026 gms. of solid per 1 c.c. which was exceeded in this respect only by the Noguchi antigen which is but artificially made to contain 0.030 grams per 1 c.c.

Its specific coefficient is from 10 to 100 times less than that of any other antigen examined. The number of units per standard amount as actually used in the Wassermann test is at least 10 to 15 times greater than that of the best antigen prepared by the wet method.

As far as its absolute specificity is concerned, this could be ascertained only by performing actual Wassermann tests on a good number of sera, using antigens prepared by both methods, moist and dry. This was done on approximately one thousand cases taken at random, the results being the following:

1. Plain extracts of the dry method compared with plain extracts of the wet method give from 3 to 5 per cent less positive fixations, with the difference, however, that such undetected positives come under the class of weak or doubtful fixations. In the majority of these cases the history and signs are against syphilis. In other words, reactions with the dry method extract are either clear cut positives or negatives with a very small percentage of doubtfuls, and nonspecific fixations are extremely rare occurrences.

2. Cholesterinized extracts of both methods, when compared together are subject to the same remarks as stated under plain extracts.

3. Plain extracts of the dry process compared with cholesterinized antigens of the wet process give results which are indicated in the following table, No. V. The percentages given are computed on 350 undoubtedly syphilitic cases under constant observation.

TABLE V  
TABLE SHOWING THE RELATIVE FIXING VALUES OF PLAIN DRY TISSUE EXTRACTIVES  
AND CHOLESTERINIZED WET TISSUE EXTRACTIVES

OF 350 UNDOUBTEDLY SYPHILITIC CASES, BY HISTORY AND SIGNS  
CHOLESTERINIZED ANTIGEN OF WET  
METHOD DETECTS AS:

ANTIG. NO. 7 PLAIN DRY DETECTS AS	Strong Positive	Weak Positive	Doubtful	Negative	Total for Antigen No. 7, Plain Dry	Difference between Antigens
Strong Positives	42 %	4 %	0 %	0 %	46 %	+ 1.5 %
Weak "	2.5 %	8 %	7.5 %	0.5 %	18.5 %	+ 5 %
Doubtfuls	0.5 %	5 %	7.5 %	2.5 %	15.5 %	+ 10.5 %
Negatives	2.5 %	6.5 %	11 %	0 %	20 %	+ 17 %
Totals for Choles- terinized Antigen	47.5 %	23.5 %	26 %	3 %	100 %	

Table V shows that strong and weak fixations are almost equally well detected by both antigens. The main discrepancy lies in doubtful and negative fixations. Cholesterinized antigen gave 26 per cent doubtful fixations and only 3 per cent negatives out of 350 undoubtedly luetic cases, while plain extractives of dry method gave 20 per cent negatives with 15.5 per cent doubtfuls.

The total difference of 17 per cent in favor of cholesterinized antigen is, however, of minor significance; because the great majority of cases that gave doubtful and negative fixations were under prolonged specific treatment. Furthermore, weak fixations with cholesterinized antigens should be taken

very reservedly. Chronic cardias, nephrities, certain acute infectious diseases as: scarlet fever, measles, pneumonia, septicemias; may at times give rise to non-specific fixations with cholesterinized antigens.

To sum up, we can state with full confidence that plain alcoholic heart extractives, guinea pig or human, prepared and standardized as outlined in the present article, are preferable and less liable to give misleading results than cholesterinized antigens such as in general use for the performance of the Wassermann reaction.

I wish to thank Dr. Max Kahn, the Director of Laboratories, for his kind interest in this work.

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## A SIMPLE DEVICE FOR THE DEMONSTRATION OF HEART BLOCK IN THE STUDENT LABORATORY\*

BY NATHAN B. EDDY, M.D., EDMONTON, CANADA

THE methods in common use in the student laboratory for the production of heart block are the Gaskell clamp, ligature of the auriculo-ventricular junction and section of the tissue between auricle and ventricle. The Gaskell clamp in the hands of students is awkward to manipulate. It is heavy and must be balanced nicely to keep it in position without pressing unduly upon the auricle. It is an additional piece of apparatus to be kept clean and in working order. It has the very decided advantage of producing little or no injury so that it can be loosened and reapplied if the first attempt is unsuccessful. Ligature of the auriculo-ventricular junction is simple but one must exercise great care to tighten the ligature without jiggling the heart lever, distorting the record. It is difficult to grade the amount of constriction secured with the ligature and attempts to loosen it are very apt to damage the heart. On this account, failure at the first attempt in many cases necessitates the making of a new preparation. Section of the tissue uniting auricle and ventricle is unsatisfactory in that it requires too fine a nicety of technic to show any gradation between normal beat and complete heart block. Obviously the damage done is irreparable.

The apparatus herein described developed in the student laboratory. In principle it was first tried by one of our students, Mr. F. E. Wait, on his own initiative. It requires no special apparatus since the parts of which it is composed are usually available in a teaching laboratory. It requires no skill

\*From the Physiological Laboratory of the University of Alberta, Edmonton, Canada.

for its successful application and a minimum of care to avoid distortion of the record. The damage done by it to the conducting pathway is slight, if any, allowing repeated trials to be made with the same preparation. It is not entirely rigid so that the auricular beat may show even when the constriction is tight.

The parts of the apparatus assembled are shown in Fig. 1. The essential part is a piece of silver wire (wire gauge number 26) doubled and run through a piece of fine glass tubing (inside diameter 2 mm.). The wire projects in a loop from one end of the tubing, making a "snare" for the heart. The ends of the wire projecting from the other end of the tubing are caught in

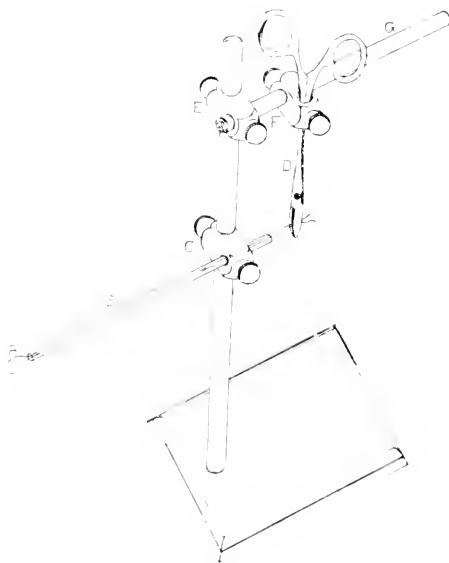


Fig. 1

a hemostat. The heart is drawn up through the wire loop as far as the auriculo-ventricular groove and then is connected to the heart lever. The glass tubing is supported in a clamp on a stativ with its smoothed end in contact with the heart. The "snare" is tightened about the heart by simply drawing the wire through the tube, slowly and steadily, as desired. The rod *G* and clamp *F* (Fig. 1) are not essential, but serve to support and steady the hemostat. The movement of the clamp and, therefore, of the hemostat is made smooth by applying a drop of oil to the rod. The clamp also makes it possible to arrest and hold the constriction attained at any time.

Tracings made with this device are shown in Figs. 2, 3, and 4. The tracing in Fig. 3 is especially of note as it is a part of a series of seven "heart







Fig. 3.



Fig. 4.

blocks" obtained in one preparation in rapid succession with complete recovery of normal sequence of cardiac activity intervening each time. Both records in Fig. 2 were made from the same heart.

This apparatus for student's use was devised for and is particularly applicable to the frog's heart. It might be possible to apply it to the turtle's heart by using heavier wire with more spring and larger glass tubing. The Gaskell clamp, however, works entirely satisfactorily on the heart of the turtle since it is easy to make simultaneous records from auricle and ventricle.

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## THE EXPOSURE OF THE CILIARY GANGLION IN THE DOG FOR USE IN EXPERIMENTAL WORK\*

BY A. R. COOPER AND J. T. GROOT, CHICAGO, ILL.

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THE literature bearing upon the pharmacology and physiology of the ciliary ganglion is lacking in clarity and precision, especially concerning the operative procedures involved in the exposure of that organ (cf. McGuigan: *Jour. Lab. & Clin. Med.*, 1920, 6 (5): 161). Therefore at the suggestion of Dr. McGuigan we have developed a method which was demonstrated at the joint session of the Pharmacological and Physiological Societies at the meetings of the American Association for the Advancement of Science held December, 1920, at the University of Chicago. And since numerous requests for information as to the exact technic used have been received we thought it advisable to present the method in detail with the hope that it might be of benefit to others.

### METHOD

The animal is anesthetized in the usual manner and then fastened upon the table on its side. It is then tracheotomized and the anesthetic administered by means of the ordinary ether-bottle. After this has been done, the common carotid artery on the side to be used is ligated in order to lessen subsequent hemorrhage, particularly in the orbit. Beginning at a point, one to two centimeters medial to the medial commissure of the eye, a curved incision is made through the scalp and extending back over the head close to the median line to the base of the ear. From the anterior end of this incision another is made at right angles to it, extending to the corner of the eye, around the rim of the upper eyelid to the zygomatic arch and thence for a centimeter or two farther downward. The flap thus marked out is reflected laterally, care being taken to secure the superficial temporal vessels situated just in front of the base of the ear. The temporal muscle thus exposed is carefully raised from the skull, beginning at the center of its upper margin and working downward in both directions, proceeding cautiously in the region of the orbit. Replace the muscle temporarily and then remove the zygomatic

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\*From the Department of Pharmacology and Therapeutics, College of Medicine, University of Illinois, Chicago, Illinois.

arch. This is done by dissecting along its upper and lower borders, sawing through its roots as far backward and forward as possible, the anterior cut coming well beneath the eyeball, and then raising it from the underlying tissues which obviously necessitates cutting through the lower end of the orbital ligament. The temporal muscle can now be easily dissected free from the coronary process of the mandible. The coronary process is then removed with bone forceps, or the jaw depressed, by stuffing a towel into the animal's mouth. Next the orbital ligament is cut away with scissors, injury to the insertions of the extraocular muscles which lie immediately beneath it being avoided.

A superficial pad of fat covering these muscles will be encountered; it is carefully dissected away with forceps, beginning well forward and working backward. Locate the insertion of the external rectus muscle; cut through it close to the sclera; holding the free end with a hemostat, carefully dissect it back towards its origin in the depths of the orbit, and sever it with a pair of

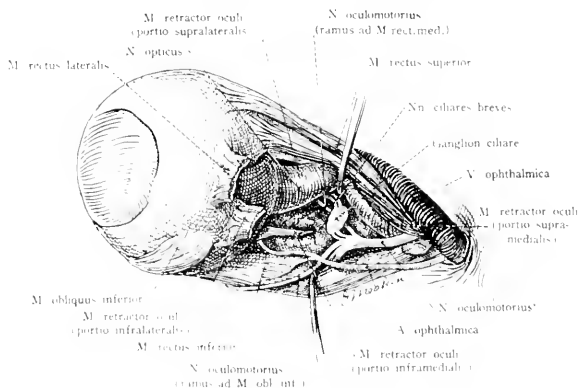


Fig. 4. Dissection showing the ciliary ganglion of the dog.

fine-pointed scissors. From now on the field of operation must be kept absolutely clear of all oozing from very small vessels which may be unavoidably cut; and in particular the ophthalmic vessels which will be constantly encountered. The ophthalmic vein is situated just behind the point where the external rectus is severed and as it is comparatively large it is very liable to be cut if sufficient care be not taken. The ophthalmic artery on the other hand is situated farther forward; it crosses the inferior rectus muscle from a lateral position posteriorly to a point beneath the optic nerve about half way along the length of the muscle. More fatty tissue which is now met with must be dissected from the surfaces of the infra- and the supralateral quarters of the retractor oculi muscle. After doing this orient the eyeball properly by replacing the insertion of the external rectus muscle in its original lateral position. In order to enlarge the field of operation the superior rectus muscle may now be removed as was the external rectus. If this be done great care

must be taken to avoid cutting the ophthalmic vein which has an even closer relation to the point of origin of this muscle than to that of the external rectus. Detach the infralateral section of the retractor oculi muscle at its insertion on the eyeball and remove it, carefully dissecting it away from the structures immediately beneath it (the ophthalmic artery in particular) and cutting it off well back. Likewise remove the supralateral section of the retractor oculi muscle which also takes origin just beneath the ophthalmic vein. A nerve will be seen crossing the anterior half of the inferior rectus muscle from a medial to a lateral position and disappearing forward under the ventral end of the muscle. This is the branch of the inferior ramus of the oculomotor nerve which goes to the inferior oblique muscle. Follow this nerve backward and medially and the ophthalmic artery will be seen to cross it about half way along the dorsal surface of the inferior rectus muscle. Just behind the ophthalmic artery the minute branches of this nerve supplying the inferior rectus muscle may be seen. Continue following this branch medially and beneath the inframedial section of the retractor oculi muscle until it is seen to unite with another branch coming from the medial portion of the orbit from underneath the optic nerve. This is the branch of the inferior ramus of the oculomotor nerve which supplies the internal rectus muscle. By gently raising the inframedial section of the retractor oculi and the optic nerve together the ciliary ganglion may now be seen in the angle between these two nerves. From the ciliary ganglion the short ciliary nerves pass dorsally and somewhat medially to the optic nerve and follow it closely to the eyeball; consequently extreme care must be taken to avoid them in the final stage of the operation. This consists of removing the inframedial section of the retractor oculi muscle and the small amount of fatty tissue in its immediate neighborhood.

The electrodes used in stimulating the ganglion should be curved at their tips and applied from the direction of the ophthalmic artery, that is, laterally, by hooking them under the structure, while the optic nerve is held to one side. By carefully examining the dorsal surface of the optic nerve the long ciliary nerve may be seen lying in juxtaposition to it in the form of two or three very delicate filaments. These may be stimulated directly or through the cervical sympathetics. If the latter is done we have found it advantageous to separate the sympathetic strand from the vagus nerve just above the inferior cervical ganglion where they are not so intimately connected as higher in the neck. In some dogs, however, the sympathetic, which is always the smaller of the two and uniformly situated laterally, may be found more or less separate from the vagus at about the middle of the neck. Examination of the heart during stimulation will indicate whether any vagus fibers are involved.

The comparatively large field afforded by this method of exposure lends itself very well to the study of the function of the sympathetic and parasympathetic mechanisms involved. Drugs may be applied directly to the ciliary ganglion and their action controlled at will; or, on the other hand, the pre-ganglionic or postganglionic fibers may be stimulated.

Finally, as pointed out by McGuigan (1, c.), the whole eye may be enucleated and the same structures stimulated as before. This is easily done by dissecting the organ away from all around the orbit, keeping close to the bone, and then cutting through the optic nerve and all of the structures surrounding it with a pair of fine-pointed curved scissors, the tips of which are inserted above the eyeball and posteriorly and downward as near the optic foramen as possible. Some distinguishing mark should be placed on the dorsal midline of the eyeball for the sake of properly orienting it after its removal. A little dissection, similar to that detailed above, suffices to disclose the ciliary ganglion and its associated nerves. Such a preparation, if kept in a shallow vessel on a pad of cotton soaked with normal saline solution and occasionally moistened, or, when not in actual use, covered by a similar pad, will keep in good condition and show contraction and dilatation of the pupil for half an hour or more.

## THE VALUE OF THE ROSS-JONES TEST ON BLOODY SPINAL FLUID\*

BY S. M. FEINBERG, M.D., CHICAGO, ILL.

ONE of the most important changes in the cerebrospinal fluid in a variety of pathologic conditions of the central nervous system is the increase in the globulin content. To determine this increase in globulin the majority of physicians and laboratory technicians employ the Ross-Jones test, this being perhaps the most useful, simple, and conservative. When a physician obtains spinal fluid, his first thought is to test for a pathologic increase in globulin, this usually giving him his first intimation as to whether the spinal fluid is normal or pathologic. Consequently, when anything interferes with the interpretation of the test so that the clinician cannot be sure in his own mind whether a positive or negative Ross-Jones test, in the particular specimen of fluid, is of any significance, then, one of the most valuable bits of information concerning that spinal fluid is lost.

This is precisely what happens when the spinal fluid is contaminated with blood due to trauma in performing the puncture; and this occurs more frequently than most of us are accustomed to believe. One authority† in his book on spinal fluid, says: "In doing any globulin test, one must make sure at the beginning, that the fluid is perfectly free of blood. Should there be any blood present in the fluid, the globulin test will be positive, even if no pathologic process exists." Other authorities express similar opinions. Consequently, we are led to believe that when one obtains traumatic blood in the spinal fluid the globulin tests should be absolutely disregarded.

However, I had frequently observed that the amount of blood in the spinal fluid, as determined by the number of erythrocytes per cubic milli-

\*From the Cook County Hospital, Chicago, Ill.

†Leyton on Cerebrospinal Fluid, St. Louis, C. V. Mosby Co., p. 153.

meter, made a difference in the results of the globulin tests. And with this observation in mind, it occurred to me that it would be of some practical value to determine experimentally the amount of blood that must be present in the spinal fluid in order to give positive globulin tests, so that when subsequently a spinal fluid is obtained that contains traumatic blood, we may be able to tell, by counting the number of red blood cells per c.mm., whether it could affect the globulin tests. A series of experiments was done using normal spinal fluid, which on previous examination had been found to have a normal cell count and negative Ross-Jones test, and later found to have a negative Wassermann. In each experiment several tubes of spinal fluid from the same case were used, and to each tube was added a definite proportion of whole blood. The results are expressed in the number of erythrocytes per c.mm. rather than as the dilution of the blood, because, although the serum-globulin is the portion of the blood which affects the results, the number of red blood cells is, nevertheless, an expression of the total quantity of blood, and at the same time, it is comparable to the test for which it is intended. The experiments were made to conform as much as possible with the actual problem in mind; consequently, very little normal saline was used in the dilution of the blood.

The technic of each experiment was as follows: Several test tubes were filled with measured amounts of spinal fluid. To the first was added 1 c.c. of blood diluted a thousand times with normal saline. Starting with tube one successive dilutions of the blood were made in the tubes so that as a result, there was in the first tube the amount of blood necessary to give 1000 erythrocytes per c.mm. of the spinal fluid, and in the following tubes 750, 500, 333, 250, 125, and 62. The resulting mixtures were used for the globulin tests. After several trials with the Pandy test it was found to be unreliable and difficult to judge, and it was decided to use the Ross-Jones test, as this appeared to be satisfactory. Over .6 c.c. of the saturated ammonium sulphate was layered .6 c.c. of the spinal fluid containing blood, and the positive and negative results noted. The results are shown in Table I.

From this table it will be seen that the results are fairly constant with several normal spinal fluids from different cases. It is evident that in none

TABLE I  
THE ROSS-JONES TEST IN SPINAL FLUIDS CONTAINING BLOOD

SPINAL FLUIDS	1 to 5000 dilution or 1000 r. h. c. per c. mm.	750	500	333	250	125	62
Alcoholic Psychosis	+	+	trace	0	0	0	0
Neurasthenia	+	+	+	?	0	0	0
Senility	+	+	+	?	0	0	0
Alcoholic Psychosis	+	+	+	0	0	0	0
Hysteria	+	+	trace	0	0	0	0
Senility	+	+	trace	0	0	0	0
Macroscopic appearance	turbid	turbid	cloudy	opal- escence	slight opal- escence	?	clear

of the cases was there a positive Ross-Jones test with the amount of blood necessary to give 250 erythrocytes per c.mm.; that in the dilution of 333 and 500 the results were variable; and that in the dilution of 750 cells or over the Ross-Jones test was uniformly positive. Following these determinations the results were applied to cases with spinal fluids which were actually bloody because of trauma and the results appeared to be of clinical value. It was also noted that a turbidity of the spinal fluid caused by the red blood cells was recognizable at a dilution of 1 to 20,000 or 250 cells per c.mm.; in other words, at a dilution too high to give a positive Ross-Jones test.

#### CONCLUSIONS

From the above it seems evident that a definite amount of blood must be present in the spinal fluid before a positive globulin test is obtained; that this amount is the concentration necessary to give in the neighborhood of a little over 300 cells per c.mm.; that when one obtains traumatic blood in the spinal fluid with a positive Ross-Jones test, if by determining the amount of blood present it is found to be below the necessary limit as here determined, the fluid is undoubtedly pathologic.

### THE TRAINING AND PROPER RECOGNITION OF THE LABORATORY TECHNICIAN

BY R. B. H. GRADWOHL, M.D., ST. LOUIS, MO.

**D**URING the past ten years there has been a remarkable development of the so called clinical laboratory. The number has become so large that it is practically impossible to man these institutions with graduate medical personnel. The reason for this is obvious: Most young physicians from whose ranks special workers are usually drawn prefer other lines than laboratory work for the development of their special faculties. Only a small number deliberately select the laboratory as a permanent career. Some enter it temporarily as a stepping stone to something else, usually internal medicine or surgery. For the most part they do not enter this field at all even temporarily. We are therefore confronted with this situation: a rapidly growing appreciation on the part of the profession for laboratory help in diagnosis; the establishment of more and more so called "commercial laboratories", which by the way is a most unwarranted and objectionable designation, the better equipment and organization of laboratories in hospitals which are taking part in the standardization plan now under way; the development of private laboratories in the offices of individuals or groups of individuals practising internal medicine or surgery; the rapid growth of public health laboratories, municipal, state and national: all this has created a demand for workers which the medical schools alone could hardly supply even were their graduates willing to enter upon this career. Inasmuch as the medical school graduates cannot and will not fill this demand, we directors



have rejoiced in the fact that we have been helped materially in our everyday routine labors by the advent of the laboratory "technician."

And who and what is a "laboratory technician"? He or she, as is more commonly the case, is a faithful ally and helper for the laboratory chief or his assistants. I shall refer to "her", as the number of male technicians is so much smaller than the female. I do not know when or where the term arose. I recall in my younger days abroad the remarkable feats to my inexperienced eyes of the so called "Diener" of the German laboratories. We might consider therefore the American laboratory technician as a trans migratory and glorified representation of the "Laboratory Diener" of the olden days. It is sufficient to say that at the present time there is a group of women (and an occasional man) who have seriously entered upon the career of laboratory technical work. Their preliminary training has been as variegated as that of the medical graduate of the pre-standard days of medical school requirements. Some have just "picked up" technic around some doctor's office or laboratory, and have little or no education and possibly have begun their medical work in the humble capacity of office attendant. Others have had a high school education and have then secured a position as a helper in some hospital or public health laboratory. More recently a number of college-bred women have prepared themselves for the work by taking up science courses and then the special medical studies of clinical pathology, the recognition of pathogenic bacteria, serology and blood chemistry. In short, just as the demand has grown, so has the supply been fairly made adequate by this entry into our ranks of these women workers. And as a better appreciation of their labors has been manifested, so a better prepared class have entered the ranks. There was a time when in referring to a piece of work, some physicians might disdainfully say, "this was done by some technician," and thereby indicate his scorn of the result and findings—that time seems happily about to pass for the reason that the despised technician is now doing excellent and dependable laboratory tests. Her reliability is in direct proportion to her training, her experience and her laboratory direction just as is the case with any other special worker in medicine. I have known women technicians who have gone the length and breadth of the land, surveying and watching the work of their masters, taking special courses with this or that man, returning to their laboratory work much improved in technic, just as is done by all workers in medicine who wish to keep abreast of the times. There is rank injustice therefore in the remarks of those who attempt to belittle the work of technicians. There are technicians and technicians, just as there are doctors and doctors. The well-trained, honest and reliable technician is of inestimable help to us and we cannot get along without her. The main question to be settled regarding the work of the laboratory technician is just how far these people ought to go in making a laboratory test and its interpretation. In my experience I have seen a number of technicians who are sufficiently well trained to conduct all of the standard tests of a laboratory, to-wit: clinical pathology, serology, blood and urine chemistry and even the later work of the laboratory designated under the title "Basal Metabolism." Some of these workers are sufficiently intelligent and well trained to procure the specimen properly from the patient and carry out every step of the procedure. There are others who

are not so well trained and their work must be closely supervised and controlled by the laboratory director, usually a man with a medical degree. The point I want to emphasize is that the *properly educated* technician is fully able to carry out the routine laboratory tests required by physicians in their everyday practice. I do not by any means wish to say that these people are qualified to interpret all of their findings. After all, the manner of interpreting laboratory findings is one that properly belongs to the medical trained laboratory worker or the laboratory trained clinical worker. It also is to be understood that my remarks concerning the value of a technician in the laboratory field, in so far as tissue diagnosis is concerned, is that they are qualified to make sections but not to make diagnoses. It is unnecessary, of course, to emphasize the necessity of a long training in pathology to equip one to be a reliable tissue pathologist.

I have found after many years of practical experience in this line that, in the main, women workers are essentially honest and conscientious, the possessors therefore of the two fundamental qualities in laboratory investigation. Leaving aside the relative dependability of the male versus female technician, we are face to face with this situation; owing to the great demand for laboratory work and the scarcity of medical talent to perform all of it, we must look elsewhere for help than to the medical school product. That "elsewhere" is the laboratory technician group. Since their work is highly satisfactory, it behooves us as medical men to whom these people come for their employment to lay down certain standards both for preliminary training and technical education which we consider fitting requirements for entrance upon serious labor of this kind. Medical examining boards reinforced by the remarkable self-purifying process undertaken in the past twenty years by the medical schools, urged and helped by the Council on Medical Education of the American Medical Association, have brought order out of chaos in American medicine and are producing a medical product called a "physician" who is really properly trained to pursue his calling with the minimum of danger to the individual citizen who from time to time is designated the "patient". The medical school problems are therefore well nigh solved; not so with the technician question.

We have no state medical examining boards for technicians. Neither have we organized schools for their training. Possibly the only well-known school in America was that initiated a few years ago by Doctor John Kolmer of the University of Pennsylvania. This we understand has been lately abandoned. These people have been given training in a few postgraduate medical schools but no school so far as I can ascertain has been conducted for the purpose of giving a woman or man a complete technical course in the subject of clinical pathology or clinical laboratory diagnosis. Nor does there exist so far as I can ascertain, any board, court or tribunal which passes upon the qualifications of these so-called technical workers. During the late war their services were much sought after for Government work. We are informed that the Army employed 398 technicians during the period of the war. Their services were eminently satisfactory according to the Army authorities. The United States Public Health Service also uses the services of technicians. They secure posi-

tions after taking the civil service examination. There are various grades of laboratory workers used in the public health services, namely; Laboratory Assistant, Junior Bacteriologist, and Bacteriological Technician. Dr. G. W. McCoy, Director of Hygienic Laboratory, Washington, D. C., states at the present time they are using about 15 technicians in that institution.

#### RECOMMENDATIONS

1. The establishment of schools under proper direction for the training of these persons.

2. The recognition of the technician of experience by some kind of board with authority to pass upon qualifications and give a certificate of merit to those who deserve it.

3. The organization by the laboratory workers *themselves* of an association for the betterment of their professional status, the purging of incompetent workers from their midst, and the matter of propaganda throughout the land among women's organizations for the purpose of enlisting the interest of women in this work, and the entrance of properly trained persons into technicians' schools.

If you will note the number of advertisements each week in the columns of the leading journals for physicians or hospitals or laboratories asking for technical help, you will gain in a measure some idea of the insistent demand for technicians. If you will also realize how difficult it is to engage the services of a technician at a distance, who has by virtue of the present haphazard system, no certificate except the letters of recommendation of her past employers, you will likewise appreciate the difficulty for a laboratory director to engage these people without a try-out which at times is very expensive and often impossible. How much better would it be were the prospective technician able to say, "I hold a certificate of ability from this or that medical board," thus simplifying the process of employment considerably. The difficulties of the doctor in California employing by letter the technician in Maine would thus be materially eliminated. At times I am overwhelmed by the number of requests for technician assistance. And even when I manage to get the address of some technician through the medium usually of classified "Want" columns, there ensues a long series of letter-writing before the two interested parties can be brought together. This I contend might well be eliminated by some sort of board, possibly some volunteer examinations conducted by a representative Bacteriologist in each one of our metropolitan cities.

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## EDITORIALS

### *The Hemoglobin Content of the Blood*

THE estimation of hemoglobin was apparently the first chemical determination in the blood to find extensive clinical application. It seems unfortunate that most of the estimations recorded should have been made employing an empirical scale with 100 as the normal, especially since the 100 is somewhat of a variable factor with different methods owing to different standardizations. Of the older clinical instruments the Fleisch-Miescher alone is standardized to give the actual percentage of hemoglobin. Sabli<sup>1</sup> states that in his original instrument the 100 was designed to correspond with an actual hemoglobin percentage of 17.2 per cent. Haldane<sup>2</sup> employs as a standard of 100 a 1 per cent solution of a blood having an oxygen capacity of 18.5 per cent. Such a blood contains approximately 14 grams of hemoglobin per 100 c.c., a quantity rather below that found in normal adult blood by most workers. Haldane states that with normal women he found an average oxygen capacity of 16.5 per cent and with children 16.1 per cent, thus necessitating a different standard of 100 for women and children.

The hemoglobin content of the blood varies widely not only in disease but also during different age periods, as recently pointed out by Williamson.<sup>3</sup>

For these reasons it is much more logical to record hemoglobin as we do other blood determinations in grams per 100 c.c., or actual per cent.

Williamson has taken great pains to definitely establish the normal hemoglobin content of human blood of both males and females for the different age periods. His figures for these different periods were made with the accurate spectrophotometric method and were the average values found on fifteen or more individuals. It was ascertained that during the first two weeks of life the hemoglobin content exceeds 20 per cent, but then drops rather abruptly about the third month to below 14 per cent and does not pass this figure until the tenth year. During the adult period of life in both sexes (From 16 to 70 years) the hemoglobin maintains a fairly constant level of slightly above 16 per cent. From the third month to the fifteenth year the values obtained in the female appear to slightly exceed the male, although from 16 to 60 the reverse is true, the hemoglobin of the female averaging close to 15.5 per cent, while in the male it reaches nearly 17 per cent.

A few observations with the same method were reported a little earlier by Meyer and Butterfield.<sup>4</sup> An average of seven normal men gave 16.6 per cent hemoglobin, while the findings for six normal women were 15.2 per cent. Since their observations were made in Germany, and agree closely with those of Williamson made in this country, it may be assumed that, for ordinary altitudes, the hemoglobin content of human blood for the adult period of life is relatively constant, being about 1.5 per cent higher in the male than in the female.

Bock<sup>5</sup> has recently made the very interesting observation that the blood plasma volume is very constant under normal and a great variety of pathologic conditions, forming about 5 per cent of the body weight. The average volume found by Bock for the whole blood of normal individuals was 8.2 per cent, while in his series of pernicious anemia cases it amounted to 5.7 per cent and in polycythemia to 13.7 per cent. Since the plasma volume is practically constant, the differences in the blood volume in these two conditions is obviously dependent upon differences in cell content, chiefly in that of the hemoglobin carrying red cells. Bock's normal cases showed a hemoglobin content (recalculated) of 16.4 per cent, whereas in pernicious anemia it was 6.5 per cent and in polycythemia 22.5 per cent.

Normally the total solids of the blood amount to about 21 per cent, and of this fully 16 per cent is accounted for by the hemoglobin. This being the case, hemoglobin is by far the most important variant in pathologic bloods. Consequently its estimation should prove of considerable value as a part of routine chemical blood analyses.

Excepting such blood diseases as pernicious anemia and chlorosis, where the hemoglobin content of the cells may be increased or decreased, respectively, thus giving rise to a high or low so-called "color index," the hemoglobin parallels fairly closely the number of red cells, and, consequently, furnishes little added information. For this reason, if one excepts the conditions mentioned, a hemoglobin estimation might more logically supplement a chemical blood analysis than a blood count.

Owing to the increased number of colorimetric methods now in common use, especially in connection with the chemical analysis of blood, a standard colorimeter (Duboseq, Kober, Bock-Benedict) has become a part of the necessary equipment of every clinical laboratory at the present time. This being the case, there is no reason why the same instrument should not be utilized for the estimation of hemoglobin. Adaptations of the Hoppe-Seyler-Haldane carboxyhemoglobin method and the acid hematin method of Sahli have already been made to these instruments. Obviously the accuracy is far greater than with the microcolorimeters ordinarily employed for hemoglobin estimation.

In the past one of the greatest difficulties in the way of making an accurate colorimetric hemoglobin estimation has been to secure a correct standard. This is now in a fair way of solution owing to the development by Van Slyke<sup>6</sup> of a simple gasometric method of determining the oxygen capacity of the blood. Van Slyke employs the same gas burette as in his  $\text{CO}_2$ -combining power estimation, and this instrument is now in nearly every clinical laboratory. As Smith, Dawson, and Cohen<sup>7</sup> have pointed out, probably some additional factors need to be considered in making an absolutely accurate calculation, but this method should ultimately furnish the basis of quickly obtaining a correct hemoglobin standard, since the oxygen capacity of blood is dependent upon its hemoglobin content. We have long known from the observations of Hüfner that one part of hemoglobin combines with 1.34 volumes of oxygen.

Another method of checking hemoglobin standards has been suggested by Berman,<sup>8</sup> viz., the estimation of its iron content. He has described<sup>9</sup> a method of estimating iron in blood which may be utilized for this purpose.

Palmer<sup>10</sup> has recently described a modification of the Haldane carboxy-hemoglobin method which may readily be made on the oxalated bloods employed in chemical analyses. He states that with this method estimations may be made in two minutes' time to an accuracy within about one per cent. The quantity of blood required (0.05 c.c.) is so small that satisfactory results may also be obtained when the usual clinical method of obtaining small amounts of blood by pricking the ear or finger tip is carried out with proper care.

When care is taken several workers have recently shown that satisfactory results may be obtained with the Sahli acid hematin method. Three serious criticisms may be made of this method as it is ordinarily carried out clinically: The standards are often inaccurate, the dilution colorimeter employed is too small to yield accurate results, and sufficient time is not generally allowed for the color to develop. Smith and Cohen<sup>11</sup> have shown, however, that with proper care quite as accurate results may be obtained with this as with the Palmer method. Robscheit<sup>12</sup> has likewise pointed out that when at least one hour is allowed for the color to develop, she was able to obtain as consistent results as with the Palmer technique. Berman<sup>8</sup> has also shown that heating for one minute will accomplish the same purpose. As a standard Newcomber<sup>1</sup> employs colored glass plates, which may be placed in the cups of the colorimeters. Although these glass standards are obviously permanent,

they have the disadvantage that each plate requires careful standardization and does not match perfectly the acid hematin in all concentrations.

It is suggested that the time-honored hemoglobin test would yield results of much greater clinical value if more attention were given to accurate standards and methods, and the results then computed to give the actual percentage of hemoglobin rather than the relation to an indefinite normal in parts per 100.

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—V. C. M.

### *Influenza and Tuberculosis—A Postscript*

A QUESTION is never trite until it is settled. Despite the formidable volume of literature which has accumulated since the influenza pandemic, dealing with the relationship between this disease and tuberculosis, the views expressed are still various and often contradictory. Much of this literature was analyzed by Vaughan<sup>1</sup> in a very thorough and critical article which appeared in this journal last autumn. What we have to say may be regarded as a footnote to his editorial.

With the advantage of a trained scientific mind, Vaughan was able to recognize the imperfect quality of the data available, and the opportunities for fallacy, and his conclusions were cautiously expressed. They were, briefly, that different observations on this subject are certain to vary, because the conditions are not constant; that phthisical patients in the mass are relatively insusceptible to influenza; but that they frequently do contract it, and when they do, it hastens their death from tuberculosis in many instances; finally, that latent tuberculosis may be activated by influenza.

Recently Fishberg<sup>2</sup> has emphatically reiterated his radical views on the subject. He writes, "We are safe in concluding that influenza has no etiologic relationship to tuberculosis." This opinion is based chiefly on the facts that the tuberculosis morbidity, judged by the demand for sanatorium treatment, and the tuberculosis mortality, judged by vital statistics, have not only shown no increase since the pandemic, but have actually decreased.

This test of morbidity, if applied to a limited section of the country, involves obvious possibilities of error. According to Fishberg, none of the sanatoria in New York are filled. In Colorado, as far as our observation goes, they

are filled, but show no abnormal congestion. The south-western states, on the other hand, are said to be crowded with tuberculous invalids; but it may be that few of these patients come from the Atlantic seaboard, and that Fishberg's observation does represent a real decrease.

Mortality statistics are more reliable, and there is no doubt that both in America and Europe, wherever nutrition is adequate, these have shown a decided decrease in the tuberculosis death rate in the last two years. Even here, however, there is danger in hasty conclusions. It has been pointed out<sup>3</sup> that a disease like chronic nephritis, even if caused by influenza, will not appear in the mortality statistics till several years later, and the same is clearly true of tuberculosis.

We, as physicians, are imbued with the text book dogma that influenza is among the chief excitants of tuberculosis, and it is difficult to rid ourselves of it. Once free of it, there is a temptation to run ahead of the evidence to the opposite extreme. For this reason it seemed desirable to consult an expert statistician, and we addressed these questions to Vice-President Frankel of the Metropolitan Life Insurance Company:

1. What is the average duration of life in fatal cases of tuberculosis, after the first definite signs of activity?

2. What, in your experience, has been the effect of the influenza epidemic in America upon the tuberculosis death rate?

His reply,<sup>4</sup> based chiefly upon data from twelve million policy holders in the Industrial Department of his Company, was as follows:

"1. This question is as yet incapable of answer. To answer this question, it would be necessary for a considerable number of years to trace the life history of patients from their earliest symptoms of tuberculosis until death. As you can see, that is a considerable undertaking and no one has thought of doing it except for very limited groups in certain sanatoria. As you probably know, this defect in the statistical situation is being remedied by the National Tuberculosis Association, which, at the suggestion of Dr. Dublin, has recently put into operation a complete plan of record keeping for all sanatoria of the country that will cooperate. This will mean that hereafter records will be available of the after-life history of the patients. Ultimately, your question will be capable of solution.

"2. It is a very interesting thing to watch the effect of the epidemic on tuberculosis. The death rate from tuberculosis rose almost at once. The rate for tuberculosis of the lungs went up to 149 per 100,000, as compared with 130 in the same quarter of the preceding year. Thereupon, the rate began to fall and the figure for tuberculosis of the lungs for the whole year was one of the lowest on record, 111.6; in 1920, the rate still further declined, reaching the unprecedented figure of 121.5. I would, therefore, say that the effect of the influenza epidemic on tuberculosis was very slight indeed. In fact, it was limited to the immediate period of the epidemic itself. After the epidemic, the tuberculosis mortality situation seems to have been greatly improved. It is, of course, possible that we are dealing with artifacts, that is, many of those who died of influenza, died in fact, of advanced tuberculosis



hastened by the influenza process. Many such cases undoubtedly occurred and were not certified as tuberculosis. This would, of course, explain some of the remarkable drop in the tuberculosis rate in subsequent periods. It is still an open question."

Another chance of error in interpreting the available figures lies in the fact that the greatest number of deaths in the epidemic fell in an age period—20 to 35—in which the death rate from tuberculosis is always high (3.5). Whether this has been of any importance in reducing the number of candidates for fatal tuberculosis we have no means of knowing—probably not, unless those who died constituted a susceptible group, but this may be the case.

It is clear that we have not, and shall not have for several years, adequate data to determine with certainty the effect of the 1918-1920 influenza epidemic upon the tuberculosis situation; and when this is settled, we shall not be certain that our conclusions will apply to the next epidemic, or to the endemic disease—so dark and foggy a field is medical science. But it already seems safe to infer, from the lack of evidence to the contrary, that the effect of the epidemic on tuberculosis was far less than we feared.

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—G. B. W. (C. T. R.)

### *Negative Wassermann Reactions in Syphilis*

THE serologic study of syphilis has become so extensive that in large medical hospitals and clinics the Wassermann reaction has become practically routine. Coincident with the development of routine tests for syphilis, clinicians have come to rely more and more upon the laboratory for their diagnosis and have neglected the methods of physical examination and analytic diagnosis. In the past when syphilis was suspected a thorough search was immediately instituted for any and all signs or stigmata suggesting this disease. How much easier it is to await the laboratory report and thereupon establish or eliminate syphilitic infection!

Not only is routine laboratory technic not infallible, but furthermore, there are without doubt not a few cases of syphilis with truly negative Wassermann reactions. These cases will be misdiagnosed if too great reliance is placed upon the laboratory examination. Pride in one's ability to recognize disease should impel one to make a thorough examination and to establish the tentative diagnosis, even before receipt of the laboratory report. If the report is then found to be at variance, it is still possible that the laboratory is in

error. It is then that judgment, that rare virtue, so necessary to the diagnostician, becomes of paramount importance.

Perhaps the condition most frequently met with, in which the patient although actively syphilitic shows a negative blood Wassermann, is chronic advanced syphilis of the central nervous system. Here the spinal fluid will usually show a positive reaction.

Marcel Pinard describes a case with multiple, painful exostoses without signs of syphilis and with a negative Wassermann and Hecht reaction, but which subsequent events showed to be a case of heredosyphilitic infection. The initial symptoms appeared at the age of 22, following trauma. As the exostoses became more numerous, syphilis was suspected and the blood was tested and found to be negative. The spinal fluid Wassermann was also negative. Under antisyphilitic treatment the symptoms rapidly disappeared.

The same author has recently reported an even more interesting case. A pregnant woman of 35, with headaches, facial paralysis and albuminuria presented herself for diagnosis. Syphilis was suspected and a Wassermann was done on three occasions, and by three different serologists, each time with negative results. The woman at term gave birth to an infant showing all of the classical signs of congenital syphilis, with skin eruption, snuffles, etc.

Two children from a previous marriage were examined and in both were found the characteristic signs of heredosyphilis.

The woman had suspected syphilis before her second marriage and had consulted a physician who made blood tests and informed her that she was "une malade imaginaire." The patient remarried, with medical sanction, and continued to produce hereditarily syphilitic children.

Pinard draws attention to the unfortunate circumstances resulting from a medical attitude of mind which depends entirely upon the result of the Wassermann reaction for guidance, and emphasizes the importance of searching for evidences of syphilis among other members of the family, a search which often furnishes information which the serologic reaction withholds.

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—W. T. V.

### *Two Recent Papers on Pellagra*

WHAT is the etiologic agent in pellagra? Is it due to an infection, or is it a nutritional disease, or do both of these agents play a part in its causation? These are questions that remain unanswered and still furnish abundant opportunity for argumentation. Among American students of this disease, MacNeal and Jobling insist that pellagra is an infectious disease; on the other hand there are Goldberger and his assistants who claim that it is a nutritional disease. Some years ago Goldberger made an experiment on eleven men in the Mississippi State Penitentiary. They were fed for some months

upon an unbalanced diet in which carbohydrates greatly and abnormally predominated. In six of these eleven patients an eruption said to be typical of pellagra developed. There has always been and still remains some question about the accuracy of this diagnosis. The result was not entirely satisfactory: it was not clear cut one way or the other. There is one thing, however, that Goldberger has demonstrated, apparently beyond question, and that is that proper feeding with an abundance of animal food both prevents and cures pellagra. The demonstration made at the great insane asylum at Milledgeville, Ga., seems to offer no other interpretation.

MacNeal<sup>1</sup> gives a very satisfactory review of the present state of the pellagra discussion. He holds that both nutrition and infection are essential in the causation of this disease. He points out that among 62,119 whites in Spartanburg County, S. C., in October, 1914, there were 1,027 pellagrins, an incidence rate of 165 per 10,000. At the same time among a negro population of 28,507 there were only 153 pellagrins, an incidence rate of 54 per 10,000. He claims that, notwithstanding the fact that the negroes live in greater poverty and have less abundant and less varied food, the incidence of the disease among them is much smaller than among the whites. Furthermore, he shows that most of the negroes who develop the disease are those who come into most intimate contact with the whites, serving as houseservants and in other relatively close relations. While pellagra is less prevalent among the negroes, it causes among them a higher death rate than among the whites. MacNeal apparently advances these statements with the idea of reinforcing his claim that the disease is infectious. Like Goldberger's experiment in inducing the disease by change in diet, we think that impartial readers will come to the conclusion that MacNeal's illustration is likewise not very convincing. It is just as easy to suppose racial differences in nutritional diseases as in infectious diseases. MacNeal, just before the war, was carrying on an experiment near Spartanburg, S. C., which was interrupted by his military service, but so far as it went MacNeal was inclined to believe that improved sanitation in a cotton village did much to reduce the prevalence of pellagra. Goldberger, studying the same village a year or two later, came to the conclusion that the improved sanitation had had no effect upon the incidence of pellagra. Studies along this line must be carried further. It is quite evident to the outsider that the contestants concerning the causation of pellagra are drawing nearer together. In the present paper MacNeal, in speaking of prophylaxis, says that the measures employed must be grouped into two classes: (1) those which enhance the individual resistance to the disease, and (2) those which exclude or diminish the opportunity for the introduction of the causative factor; in other words, this author evidently believes that the infecting agent causes the disease only among those who are nutritionally below par. On the other hand, Goldberger does not deny the possibility of infection, though he quite justifiably regards this as very remote. He and his assistants have inoculated themselves with every possible secretion and excretion of pellagrins and have not induced the disease. It might have been otherwise, how-

<sup>1</sup>Am. Jour. Med. Sc., 1921, clxi, 470.

ever, had they reduced their resistance before these inoculations were made, by living upon an unbalanced diet. The importance of nutrition in many of the infectious diseases is being more fully appreciated, and we are attaching to it more importance than we did. Whatever may be the ultimate verdict as to the etiologic agents in this disease, it is quite certain that by a properly balanced diet primary cases may be avoided and recurrences may be prevented.

The second paper on pellagra is by Sullivan, Stanton and Dawson<sup>2</sup> and concerns itself solely with the question of metabolism in pellagrins. Their results are substantially as follows: (1) Mineral metabolism as measured by the  $P_2O_5$  elimination is low, even when a generous diet with an abundance of milk is given. (2) Putrefactive processes in the intestines are abnormally high. (3) Albumin and casts are found in the urine in about fifty per cent of cases, although there may be pellagra without change in the kidney. (4) The total nitrogen excretion is low; especially is this true of the urea nitrogen, suggesting insufficient activity of the liver. (5) The ratio for ammonia nitrogen and undetermined nitrogen is high. (6) The excretion of uric acid and creatinin is low. (7) The utilization of protein food, even after several weeks on a remedial diet, is subnormal. (8) After a month or more on the curative diet, urinary ingredients rise to approximately normal amounts. This is especially true of urea which rises to the normal, while the ammonia ratio falls to the normal.

—V. C. V.

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<sup>2</sup>Arch. Int. Med., 1921, xxxvii, 287.

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## *ORIGINAL ARTICLES*

### APPARATUS USED IN THE ESTIMATION OF BASAL METABOLISM\*

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THE increasing interest in basal metabolism as a means of diagnosis has raised many questions as to the type of apparatus most suitable for this test. The clinician naturally craves a small portable apparatus which can be carried from house to house and used with the same ease and accuracy as his blood-pressure apparatus and his clinical thermometer. Unfortunately this demand can at best be but partially satisfied, as all appliances so far devised involve the handling and measurement of gases; this includes the vexing problem of corrections for temperature, barometric pressure, and water vapor,—factors with which the clinical observer has never had to contend.

In designing apparatus for clinical respiratory work, portability seems to have become the prime requisite. While the idea is fascinating, in reality nothing detracting from the accuracy of the apparatus should be sacrificed on this score. The patient who is too ill to be moved to a laboratory, or to the physician's office, is not the type of case in which basal metabolism estimations are of value. Those using portable apparatus rarely transport them: almost invariably the test is done in the clinician's office or, in the case of a hospital, a suitable room is selected and the patient brought there for the test.

Basal metabolism is an expression of the heat production of the body while completely at rest and in the post-absorptive state, such a condition as is found upon awakening in the morning. The brilliant and painstaking work of Voit, Pettenkofer, Atwater, Benedict, Lusk, DuBois, and their many coworkers has demonstrated that the heat production can be indirectly determined by measuring the oxygen consumption, the heat value for the latter being derived from the respiratory quotient.

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It is necessary, therefore, to determine the amount of oxygen consumed and the amount of carbon dioxide produced in a given time. To accomplish this, two methods are in vogue. In the "open-circuit" method, the subject inspires atmospheric air and expires into a gasometer through a series of tubes, mask, and valves. The expired air, having been measured and its volume corrected, is analyzed for carbon dioxide and oxygen; the amount of oxygen absorbed is calculated on a basis of the ratio of expired nitrogen to atmospheric nitrogen. Dividing the volume of carbon dioxide produced by the oxygen absorbed gives the respiratory quotient, which has a known heat value for each liter of oxygen. In this manner the total calories produced per hour may be calculated. Accepting the heat production as practically proportionate to the surface area determined by the DuBois and DuBois formula,<sup>1</sup> the calories produced may be expressed in terms of calories per square meter of body surface per hour. This result, the basal metabolism, is usually reported as the percentage above or below the average of the DuBois normal standard.

The "closed-circuit" method requires the subject to rebreathe from a circulating current of confined air, rich in oxygen. The carbon dioxide and water vapor are removed by suitable absorbers and the consumed oxygen is represented by the decrease in volume, or by the weight of oxygen required to maintain the original volume. The carbon dioxide produced can be determined by weighing the absorbers, and the remaining calculations are the same as in the open-circuit method. This principle has been used by Benedict<sup>2</sup> in developing his "clinical respiration apparatus" and later, the "portable respiration apparatus." It has also been applied in simplified apparatus as those of Jones,<sup>3</sup> and Sanborn;<sup>4</sup> in these appliances the carbon dioxide is removed by absorbers and the consumed oxygen determined directly by the decrease in volume of the rebreathed air, which is almost pure oxygen. The carbon dioxide is not measured. The respiratory quotient is assumed to be 0.82, giving a constant heat value for oxygen.

In equipping the respiration laboratory of the New York Post Graduate Medical School and Hospital, we were greatly influenced by the broad experience of Boothby and Sandiford in the clinical application of the test. Their arguments in favor of the open-circuit or gasometer method for institutional work seem to be sound, and, despite the bugbear of gas-analysis, the method is eminently satisfactory. In this procedure the expired air is collected under the most favorable conditions. The subject reclines in a pleasantly furnished room adjoining the laboratory which contains the entire apparatus; the face mask is adjusted, and the test started after the gasometer has been completely washed out with expired air. The test is started and stopped during inspiration without the patient's knowledge, and without his undesirable cooperation. No skill is required in this part of the procedure and, once the apparatus has been tested, the danger from leaks is negligible.

After the expired air is collected and measured in the gasometer, multiple samples can be kept in suitable containers and the gas-analyses repeated, even after an interval of several days, if there is any question of the accuracy of the results.

By the use of Boothby and Sandiford's calculation sheets<sup>5</sup> each step in the procedure can be checked by a second assistant, thereby greatly decreasing the chances of technical error. Logarithms, whose name alone has probably inspired dread in the minds of the uninitiated, is an invaluable means of accurately carrying out routine calculations. The use of the tables for multiplication and division can be mastered at one sitting, and it is greatly to be regretted that this valuable aid is not in the hands of every one attempting to do scientific work. Employing the Haldane apparatus<sup>6</sup> or one of its modifications,<sup>7</sup> the determination of the carbon dioxide is rapidly carried out before the oxygen analysis. With but slight additional work we are given a much broader view of the metabolic processes, the actual respiratory quotient is determined, the true heat value of the oxygen known, the basal metabolism most accurately estimated and fields thrown open for intensive studies in metabolism. Benedict<sup>2</sup> has stated that "only that form of apparatus which permits exact determinations of the respiratory quotient as well as of the total metabolism, need be seriously considered for the best clinical work."

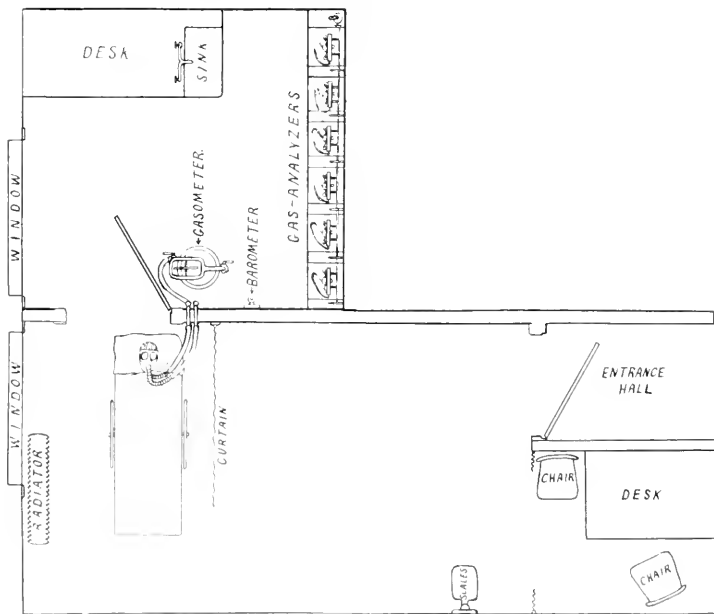
The accuracy of the gasometer method is unquestioned, it is chiefly criticized for its lack of portability and for its time-consuming gas analyses which place it far beyond the reach of the great majority of physicians. This is quite true, but it is equally true of complement-fixation tests, the colloidal gold test, chemical analysis of the blood, and many other laboratory procedures, all of which are time-consuming and require special skill not possessed by the average clinician. The chief use of basal metabolism tests at the present time is in the diagnosis and prognosis of thyroid disease; the result of the test frequently determines when serious operative procedures can be undertaken with least risk for the patient,—certainly time and labor are paltry considerations under the circumstances. Boothby and Sandiford<sup>5</sup> have shown that the technique of gas analysis can be acquired by any intelligent high-school girl, and that the time required for the test is well within one hour. The entire procedure can be carried out by a well-trained technician under proper supervision. The "closed-circuit" method, when the respiratory quotient is determined, is an accurate means of estimating the heat production, and in skilled hands has resulted in the accumulation of valuable scientific data. Its use in routine institutional work, however, calls for extreme care and attention, first in testing the apparatus for leaks and secondly in making the determination. The oxygen absorbed is represented by any change in volume of the enclosed air; leaks in or out of the apparatus, incomplete absorption of carbon dioxide, and retention of air in the lungs or stomach at the beginning or end of the test will directly affect the results. Any check on the accuracy of the determination can be made only by again subjecting the patient to the test, and any check on the observations must be made by a second observer at the time of the test. It would seem that the time and skill required to conduct a number of tests with this type of apparatus greatly exceed that of the open-circuit type, while the difficulty in checking the observations renders it less desirable in routine institutional work.

The simplified types of closed-circuit apparatus<sup>2, 3, 4</sup>, in which the only

estimation is that of oxygen consumption, doubtless have a wide field of usefulness, but this field should be closely defined and radical attempts, either medically or surgically, to correct the patient's metabolism should be instituted solely on the most careful observations using the most accurate method available. The simplified appliances have been introduced to the clinician as useful aids in diagnosis, but it is questionable whether they should be used as a means of surgical prognosis, or of conducting original research in metabolism.

RESPIRATION LABORATORY OF THE NEW YORK POST GRADUATE  
MEDICAL SCHOOL AND HOSPITAL

In planning a new department for a large institution, one soon discovers that space is the deciding factor, and that one must design and assemble the



equipment with this limitation ever in mind. Our experience in this undertaking was no exception to the rule, but the very restriction imposed proved to be a blessing in the end, as it resulted in the assembling of a very compact and workable outfit for metabolism estimations. In general, the apparatus and mode of procedure do not differ from those frequently described,<sup>1,2,3</sup> but in the particular design and assembling of the appliances, innovations have been



introduced which add to the accuracy and ease of manipulation, and seem to afford a sufficient excuse for a detailed description at this time.

Having decided upon the gasometer method as being the most suitable for routine institutional work, the first consideration was the handling of the patients; this must be done expeditiously, and in such a way as to allay their fears and to permit of complete bodily rest for a period of at least half an hour before the test is started. Two small rooms were placed at our disposal, the smaller, measuring 8 ft. by 8 ft. was set aside for a laboratory, the larger, 8½ ft. by 20 ft., was selected as an office and rest room for the patients. This room is finished in French gray with blue draperies and the hospital atmosphere is avoided as much as possible. Couches for the patients were dispensed with in favor of adjustable wheel-chairs, the latter, when comfortably padded prove very satisfactory, as the patient can remain in one chair from the time he leaves his room until his return at the end of the test. In



Fig. 2.

our rest room, three patients can be accommodated at one time, the chairs being moved into position for taking the test without disturbing the occupant. The general arrangement of the rooms is shown in the accompanying plan (Fig. 1).

It will be seen that with the exception of the mask and short lengths of air-tubes, the entire apparatus is placed in the laboratory. The door, which

contains a small observation window, affords the observer easy approach to the patient, so that one person can manipulate the gasometer and at the same time observe the patient.

#### MASK

The large number of breathing appliances, which have been introduced for use with respiration apparatus, is an evidence of the difficulty experienced in finding something suitable for this work. Some device is needed which can be rapidly fitted to any patient, which will permit of his normal type of respiration, which will accurately conduct the inspiratory and expiratory air without offering resistance, and which can be worn in comfort and without the danger of leaks. The gas mask<sup>9</sup> of the French army fulfills all of these requirements, when used in conjunction with the rubber flutter-valves introduced by the British during the late war. The mask (Fig. 2) is made of thick rubber, covers the whole face, and presents broad surfaces which closely

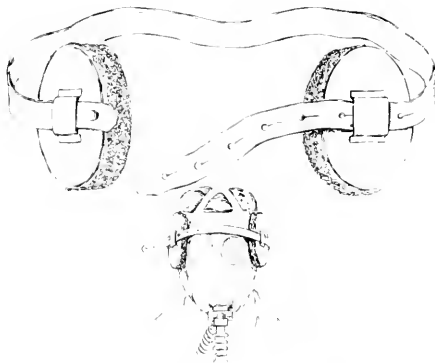


Fig. 3.

engage the forehead, sides of the face, and jaw. The tissues in these regions are well supported by the bony framework of the face and the mask readily adapts itself to these fixed surfaces. It is held in place by elastic straps passing around the head. With emaciated subjects, leaks may occur above or below the zygoma, in this area the pull of the straps is in the same plane as the surface of the face. In such instances, the leaks are readily overcome by placing 5-inch rubber sponges over these areas of the mask and binding them in place with a 3-inch bandage. In place of a bandage for this purpose, the sponge holders shown in Fig. 3 are more satisfactory; the sponges are cemented to thin metal plates which are adjustable on a broad strap passing around the head. In this mask the incoming air is directed upward towards the windows, the opening of the expiratory tube being opposite the nose and mouth; this insures complete ventilation of the space and no discomfort results. When applied to some individuals, the chin pad permits the mask to

press on the throat; this can be readily overcome by drawing the mask away from the chin by means of a cord fastened to the tube-connections on the mask, and tying the cord to a hook directly over the subject's head (*D*, Fig. 4). With this cord the mask can be comfortably adjusted and it has the added value of preventing movement on the part of the patient. Following the test, the mask and attached tubes are removed from the pipes (*C* and *E*, Fig. 4) passing through the wall, and are readily cleansed with soap and water.

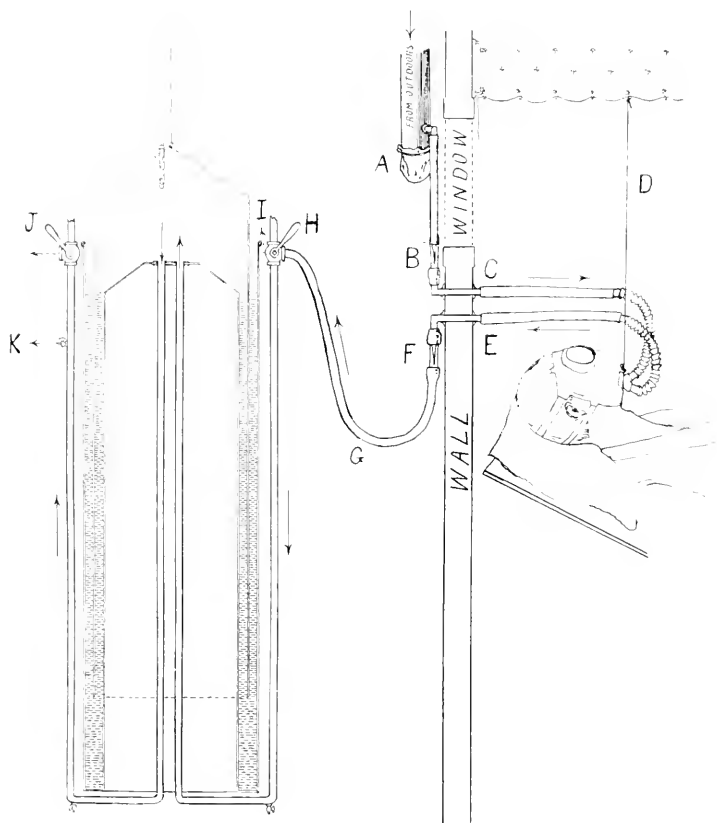


Fig. 4.

## PIPES, VALVES AND CONNECTIONS

Outdoor air is brought to the laboratory through a 4-inch galvanized sheet iron drain pipe. This pipe terminates 2 feet above the valves, the open end being closed by a rubber bathing cap (*A*, Fig. 4) held in place by a rub-

ber band. The cap takes up the pressure of gusts of wind and prevents its blowing through the valves. A 2-foot length of 24 mm. rubber tubing leads from the large pipe to the inspiratory valve; this is for the purpose of trapping any expired air which might backlash during expiration. The arrangement of the valves is shown in Figs. 4 and 5: they are enclosed in flattened glass cases which are mounted directly on the laboratory ends of the wall tubes (*B* and *F*); the latter have elbows directed upward on the inspiratory and downward on the expiratory,—this permits both the flutter-valves to hang



Fig. 5.

downward, in which position they functionate most favorably. From the lower end of the expiratory valve-case, a 24 mm. rubber tube (*G*) leads to the gasometer.

#### GASOMETER

The gasometer (Fig. 6) is designed as a stationary piece of laboratory apparatus; its ball castors, however, permitting it to be moved about the room should this be desired. For clinical purposes a gasometer of 90 to 100 liters capacity is the most serviceable. As the volume is measured by the rise of the gasometer bell, it is evident that for a given volume, the longer the

bell the more accurate the reading. The bell of this apparatus is 100 cm. in length and has a diameter of 34.59 cm.; measuring the rise of the bell to the nearest half-millimeter, a change in volume of 47 c.c. can be detected. The

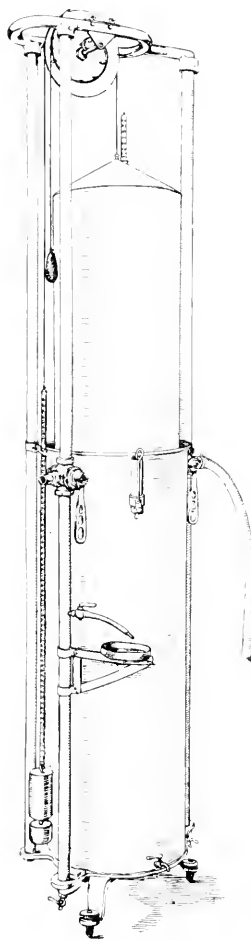


Fig. 6.

apparatus is 8 feet high and occupies no more floor space than an ordinary chair. The framework is rigidly constructed of iron pipes and castings, at the top it supports the counterbalance wheel, on its flat base rests the copper water-bath. The base consists of a malleable casting containing inlet and outlet passages for air; it is fitted with three adjustable legs for leveling the apparatus, and is threaded to receive the supporting and air pipes of the framework. Extending upward from the center of the base and connected with its air passages are two one-inch pipes which pass through holes in the upper end of the obturator of the water-bath the joints being made firm and airtight by means of plates. The air passages are continued as part of the framework to a height of 4 feet where three-way valves are placed; from these the piping extends to the casting forming the upper part of the framework. The grooved counterbalance wheel is of brass and has a diameter of 23 cm.; it is mounted on a steel axle with spindle bearings, the latter being adjustable on the upper casting of the framework. The wheel carries a grooved spiral from which is suspended a compensating weight; as the bell rises from the water and becomes progressively heavier, the leverage of this weight is correspondingly increased and in this way the bell is exactly counterbalanced in all positions.

The bell is suspended by a piano wire which passes in the groove of the wheel and is continuous with a 100 cm. length of steel measuring tape, to the lower end of which the main counterweight, which is in two sections, is fastened by means of hooks. The tape, which reads in millimeters from below upward, passes through a suitable guide bearing a pointer at which the readings are made. The bell and water-bath are made of copper. The water-bath is circular in plan and has outer and inner walls, 4 cm. apart; the outer wall is 110 cm. high; the inner wall has a height of 100 cm. and with a truncated conical roof forms the obturator of the bell. The water-bath has a drainage cock near the bottom and a constant water-level attachment fastened to the

outer wall at a height of 100 cm. In use the bath is kept filled with water to this level. The outlet pipe has a sampling cock placed about a foot below the three-way valve; this pipe also supports a shelf for the sampling bottles. The bell carries a thermometer for recording the temperature of the air.

#### COLLECTION OF EXPIRED AIR IN GASOMETER

The patient is placed in a semi-reclining position in a wheel chair and is allowed to rest for twenty minutes or longer before the test is started. During this period the respirations and pulse rate are closely observed and the mask is not applied until the patient appears to be mentally and physically at rest. At this time one ascertains that the body temperature is normal. While the subject is resting one empties the gasometer by removing the lower portion of the counterweight and turning the valve *J* (Fig. 4) to communicate with the room air; the bell sinks until it rests on the obturator and the pointer will stand at zero on the measuring tape. The valve *H* (Fig. 4) is now turned so as to connect the air-tube (*G*) with the gasometer. The rubber cap (*A*) is removed for a few minutes, permitting the large intake pipe to fill with fresh air from outdoors; the cap is then replaced.

When the patient is sufficiently rested, the ends of the tubes attached to the mask are dipped in water and slipped over the brass pipes perforating the wall at *C* and *E*, care being taken that the inspiratory pipe leads to the upper openings in the mask. The mask is then applied by holding the chin portion in position and pulling the straps over the top of the head. When accurately fitted, the mask-cord (*D*) is tied to the overhead hook, holding the mask and head in the most comfortable position for the patient. From the time the mask is applied, the inspiratory air is drawn from outdoors through the pipes to the flutter-valve (*B*) and thence to the mask. The expiratory air passes from the mask, through the flutter-valve (*F*) and along the tubing to the small space between the top of the bell and the obturator; from there it passes through the outlet pipe and escapes into the room through the valve (*J*). In this way the entire dead space of the apparatus is flushed out with expired air.

As an extra precaution, the lower portion of the counterweight is replaced, at the same time closing the valve (*J*). The expirations are now caught in the gasometer. When the bell has been elevated 6 or 8 cm., the valve (*H*) is turned during inspiration, cutting off the gasometer and permitting the expirations to escape through the vent (*I*). The bell is now dropped nearly to the zero mark by removing the lower counterweight and opening the valve (*J*). This valve is then closed, the counterweight replaced, and the reading on the tape recorded to the nearest half-millimeter. One is now ready to start the test. The respiratory phases can be readily followed by observing the flutter-valves (*B* and *F*). During the inspiratory phase, the valve (*H*) is quickly turned to communicate with the gasometer and at the same time one presses the stopwatch and the collection is begun. The observation window permits the attendant to closely watch the subject during these procedures and record any movements. While the air is being collected, the respiration and pulse

rates are recorded at frequent intervals, and the patient is admonished to remain very still and encouraged by explanations that the test is nearing completion and that everything is very satisfactory. The duration of the test is determined by the subject's rate of ventilation. As a rule the gasometer is nearly filled at the end of 10 to 12 minutes, although this period may vary from 5 to 15 minutes. Calculation is facilitated by stopping on the minute, the quarter, or the half minute. This is accomplished by holding the stop-watch near the inspiratory valve (*B*) in such a way that both can be observed at the same time; the other hand grasps the valve lever (*H*): at the selected period of time, and during inspiration, the valve (*H*) is quickly turned cutting the patient off from the gasometer. Should the subject be expiring, one waits until expiration is completed before closing the valve. The watch is stopped at this time. One must avoid shifting this valve during expiration as it is very disconcerting to the patient. The reading on the steel-tape is now recorded to the nearest half-millimeter. The difference between this and the

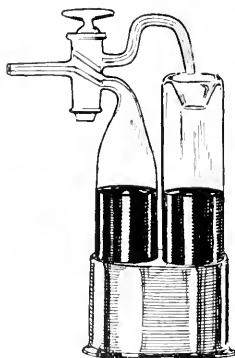


Fig. 7.

first reading tells the height in centimeters to which the bell has risen. As the bell is exactly counterbalanced in all positions, the enclosed air is under the prevailing atmospheric pressure. The lower portion of the counterweight is now removed leaving the air under positive pressure.

Since volumes of gas vary directly with the temperature and inversely with the barometric pressure, it is necessary to record these factors which will be used later in the calculations. The temperature is determined by a Centigrade thermometer inserted in the bell. A brass-scale mercurial barometer is used in recording the atmospheric pressure.

Samples of the expired air can now be collected in gas-sampling bottles.

#### GAS-SAMPLING BOTTLES<sup>10</sup>

The gas-sampling bottle (Fig. 7) consists of parallel glass cylinders communicating at the bottom through a small opening. The cylinders are firmly cemented into a metal case which forms a base. The top of one of the cylinders

has a funnel-shaped inversion; the other tapers off to a 2 mm. capillary tube to which a 3-way stop-cock is fused. The second opening in the stop-cock communicates with a capillary tube which bends over the inverted top of the first cylinder. The stop-cock terminates in a straight spout of capillary tubing 3 cm. long. The appliance is half filled with mercury, and before use is flushed out with 1 per cent sulphuric acid. A 6 cm. length of 3/16 inch rubber tubing is permanently attached to the spout.

In preparation for use, one sets the stopcock so that the spout communicates with the gas chamber (Fig. 7). The bottle is now tilted forward over a

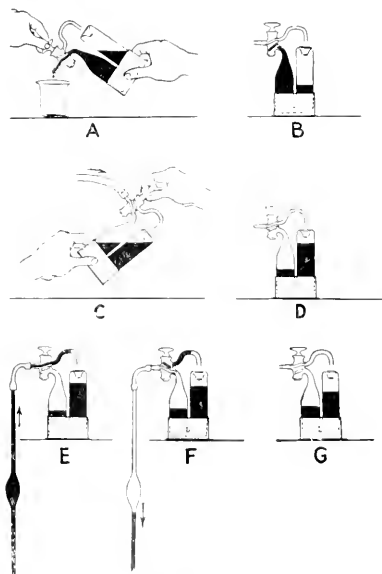


FIG. 8.

small beaker containing 1 per cent sulphuric acid, into which the rubber tube dips. The contained air is forced out by the mercury and when the bottle is tilted backward, acidulated water is drawn into the chamber. The apparatus is again tilted forward, this forces out the air and water. This position is maintained until mercury drops into the beaker (A, Fig. 8). The stopcock is immediately reversed; this leaves the gas chamber and the lower lead of the stopcock filled with mercury (B, Fig. 8) and the spout is in communication with the bent capillary tubing through which the air to be sampled is blown. Enough acidulated water remains in the chamber to saturate the air when the bottle is filled.



## COLLECTION OF AIR SAMPLES FROM THE GASOMETER

Having removed the lower portion of the counterweight following the final reading of the gasometer, the enclosed air is under positive pressure. The prepared sampling bottle is now placed on the shelf and connected with the sampling-cock (*K*, Fig. 4). This cock is now opened and the air blows through the curved tube of the sampling bottle. Half the air in the gasometer is allowed to escape through the valve (*J*, Fig. 4); this is to insure a fair sample, as recommended by Boothby.<sup>5</sup> The stopcock of the sampling bottle is now reversed, the bottle removed from its shelf, and tilted backward (*C*, Fig. 8) and the compartment fills with the air from the gasometer. The stopcock is returned to its original position and the sample is trapped under pressure in the bottle (*D*, Fig. 8). Before trapping the sample, it is sometimes

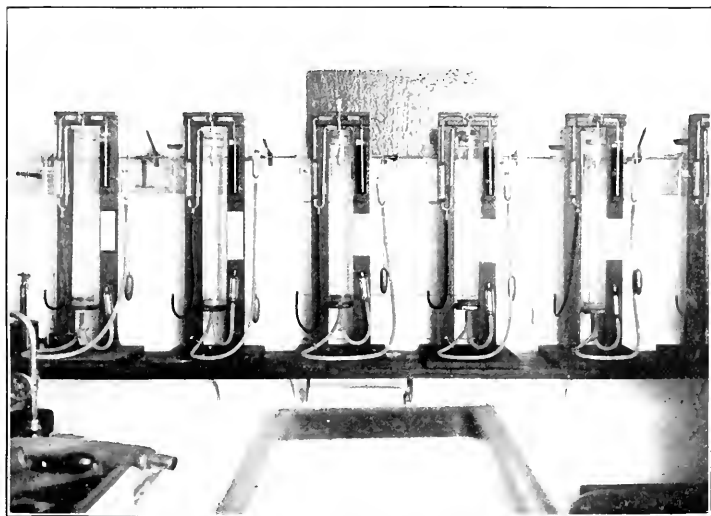


Fig. 9.

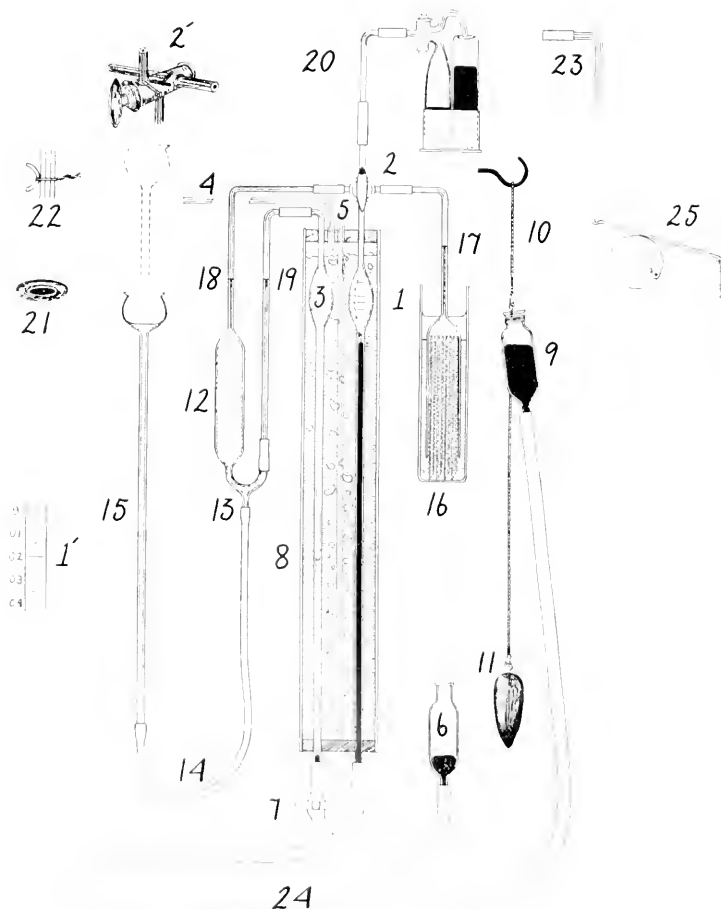
advisable to tilt the bottle backward and forward several times, at the same time allowing air to escape through the valve (*J*, Fig. 4); in this way one is certain of securing a good sample of the enclosed air. Two or three bottles can be filled in this manner, each bottle holding enough air for six analyses.

## PRINCIPLES OF GAS ANALYSIS

The Haldane method is used in determining the amount of carbon dioxide and oxygen in the expired air. In principle, a volume of air is drawn into a graduated burette, where it is saturated with water vapor and measured; the air is then passed back and forth into a potash pipette, where the carbon dioxide is removed; it is returned to the burette and again measured, the difference

between this and the first reading representing the volume of carbon dioxide. The oxygen is removed in a similar manner by passing the air into a second pipette containing a potassium pyrogallate solution; after the oxygen is absorbed, the gas is returned to the burette and measured, this second loss in volume representing the oxygen in the sample of air.

In applying this method, several modifications of the original parts have been incorporated in the apparatus, the principal changes being the adoption



of Henderson's burette<sup>7</sup> with a four-way stopcock, and that author's type of oxygen absorber. These greatly simplify the assembling and cleansing of the parts and permit one to change the pyrogallate without disconnecting the apparatus.

#### DESCRIPTION OF APPARATUS FOR GAS ANALYSIS

The complete apparatus is pictured in Fig. 9, and shown diagrammatically in Fig. 10. It is mounted on a stout wooden support (Fig. 11), having grooves to receive the tubing and six brass clamps, by which the glass parts are held in place. On the top of the frame is a brass shelf upon which the gas-sampling bottle is placed. The space behind the water-bath is closed with ground glass, and behind this is placed a 12-inch "show-case" electric light bulb in a chain-operated socket, the chain being brought to the front of the support, so that the light can be readily switched on and off during the analysis. The table of

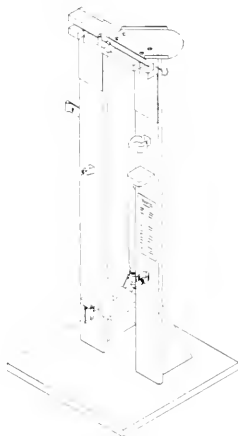


Fig. 11.

corrections for the burette is glued to the support to the right hand side of the ground glass. The frame carries a hook at the upper right hand corner, from which is suspended the mercury reservoir and its lead counterweight.

The assembled apparatus ready to be placed on its support, is shown in Fig. 10. It consists of a 10 c.c. burette graduated in hundredths (1) having a four-way stopcock at the top (2); this, along with a control tube of the same volume (3), is enclosed in a water-bath extending the full length of the burette. The water-bath is closed at top and bottom by rubber stoppers, through which the burette and control tubes protrude. The upper stopper has two additional perforations, one of which carries a suction tube (4) to the air space above the water, the second carries a tube (5) open at the top and extending to the bottom of the bath. When the first tube is connected with a suction pump, air is

kept constantly bubbling through the water, keeping it stirred up and equalizing the temperature of the enclosed burettes.

From the lower end of the control tube, a 24 cm. length of pressure tubing leads to a mercury reservoir (6). This tube carries a Hoffmann improved clamp (7) by which the lumen is occluded when the fluid in the control tube is set at the proper level (8). Attached to the lower end of the burette is a 95 cm. length of pressure tubing continuous with a mercury reservoir (9) which is suspended from the hook by a braided sash-cord (10) and counter-balanced by a conical lead weight (11). This weighs 447 gms. and exactly balances the reservoir and tube filled with mercury when the latter is half-way up the burette. When the reservoir is raised or lowered, there is enough friction between the cord and hook to hold the mercury at any level. For fine adjustment of the mercury level, the cord is grasped with the finger and thumb and rolled over the hook as one would turn a milled wheel.

The potash pipette (12) is placed to the left of the water-bath. Capillary tubes lead from the top and bottom of the pipette respectively to the left lead of the stopcock and to the upper end of the control tube. All the connections are made glass to glass and held tight by 4 cm. lengths of pressure tubing. The lowest opening in the pipette (13) is connected with a 21 cm. length of 3/16 inch sulphur-free black seamless rubber tubing (14). This is attached to the lower end of the thistle tube (15) which serves as a reservoir for the potassium hydroxide solution. This tube slides through a clamp on the frame, and by raising or lowering the reservoir equal changes of pressure are produced in the burette and in the control tube. The thistle tube is covered with a rubber cap (21) having a small perforation to permit the passage of air.

The right lead from the stopcock is continuous with a capillary tube which terminates in an inverted bell enclosed in a flat-bottomed tube (16). This bell is loosely filled with lengths of 3 mm. thin-walled glass tubing, beveled on the ends which rest on the bottom of the tube. When in use, this tube holds potassium pyrogallate covered with a 2 cm. layer of petroleum oil, which protects it from the air. This part of the apparatus constitutes the oxygen absorber.

The capillary tubes leading from the absorbers serve as manometers in adjusting the apparatus. A permanent mark (17) is cut in the tubing of the oxygen absorber 5 cm. above the bell. Sliding markers, as described by Henderson, are conveniently used on the manometer tubes leading from the CO<sub>2</sub> absorber (18, 19). These are readily made by twisting a loop of fine brass wire around the tube, along which is laid a short length of a rubber band. This marker (22) is easily moved to the required position by grasping the ends of the rubber and the twisted wires; the rubber between the wire and the glass holds it firmly in position.

The upper lead from the stopcock is connected by pressure tubing to an elbow of capillary tubing (20), the horizontal part of which is 21½ cm. long and is at a height of 13 cm. above the shelf upon which the bottle rests.

In the construction of the apparatus, the capillary tubing used should be of uniform bore throughout and should be not less than 1.1 mm. and not

greater than 1.3 mm. in diameter. Care should be taken that constrictions do not occur where the tubing is joined to the bulbs, these retain fluid and interfere with the proper working of the apparatus. The burette, as supplied by the manufacturers, has a stopcock fused on the lower end. After recalibration this is cut off and the end of the burette smoothed with a fine file under water. The ends of the capillary tubing should be ground in this manner rather than fire-polished. The method of recalibrating the Haldane burette is completely described by Boothby.<sup>5</sup> The same instructions apply to the Henderson type, excepting the calculation of the mercury in the stopcock, which is not included in the volume of this burette. Before assembling the apparatus, the glass parts should be thoroughly cleansed with soap and water, then with warm cleaning solution, and finally rinsed in distilled water.

All the rubber tubing used in the construction of the apparatus should be scrubbed in soapy water in order to completely remove the tale. This can be done by coiling a pipe-stem cleaner, fastening it to a long wire and drawing it back and forth through the tubing; the rubber is then carefully rinsed and flushed out with one per cent sulphuric acid. In assembling the apparatus, a small amount of vaseline may be used in slipping the rubber over the glass tubing; this makes a very tight joint as it causes the rubber to adhere. In taking down an apparatus assembled in this manner, one must cut off these connections with a knife.

Black rubber grease is used on the stopcock. This is prepared by heating together equal parts of finely cut black rubber tubing, and lanolin. When the rubber has completely dissolved, the mixture is poured into an ointment jar and kept covered.

In preparing the absorbing solutions, it is convenient to keep a stock saturated solution of potassium hydroxide. For use in the potash pipette, this is diluted to a specific gravity of 1.15. The potassium pyrogallate solution is made by diluting the potassium hydroxide solution to a specific gravity of 1.55, and adding 10 gm. of pyrogallie acid to every 100 c.c. of the dilution. This absorber should be made up in quantity and is conveniently kept, well stoppered, in small citrate of magnesia bottles; the acid is first placed in the bottles, the potassium hydroxide solution added, and the bottles stoppered at once, making sure that the rubber washers are well in place. The solution improves with age. If one must use a freshly prepared solution, its efficiency as an absorber can be increased by exposing it to the air.

#### MANAGEMENT OF APPARATUS FOR GAS ANALYSIS

*Preliminary.*—Fill the water-bath with distilled water to within one inch of the upper stopper. Release clamp (7) at bottom of control tube. Place 2 c.c. of 1 per cent sulphuric acid in the control tube reservoir (6). Hold reservoir so that the dilute acid can barely be seen above the rubber tubing and superimpose about 6 c.c. of mercury,—this causes the acid to rise in the control tube. By raising or lowering the reservoir the acid can be brought to the level (8) in the control tube. This level is at the height of 8.5 c.c. on the burette. Close clamp (7) and replace the reservoir in its support.

Remove the barrel of the stopcock. Lower the mercury reservoir (9) until its opening is opposite to the bottom of the water-bath, and fill to the neck with mercury. Place the thistle tube (15) at a level slightly above that shown in the diagram and fill the pipette (12) with the dilute solution of potassium hydroxide until the level in the thistle tube is opposite to the mark (18). Cover the tube with a rubber cap (21). Fill the oxygen absorber (16) with the solution of potassium pyrogallate to a level with the top of the bell, and superimpose a 2 cm. layer of petroleum oil.

Grease the barrel of the stopcock with the black rubber mixture, care being taken that the holes are not occluded. Place the barrel in position and turn with gentle pressure until the burette is in communication with the intake tube (20). The black mark\* on the stopcock handle indicates the lead which is in communication with the burette. Attach glass elbow (23) to the intake tube (20) so that the former points downward, and dips into dilute sulphuric acid contained in a beaker held beneath the tube. The burette is now flushed out with the acid by raising and lowering the mercury reservoir (9). The burette is then emptied by raising the reservoir until a few drops of mercury fall into the beaker. Remove the glass elbow (23).

The volume of air in the control tube is adjusted so that, when the meniscus is at the mark (19), the acidulated water in the control tube will be at a level (8) opposite to the 8.5 c.c. mark on the burette. This is accomplished by releasing the clamp (7) and raising the reservoir (6), causing air from the control tube to escape into the pipette (12). This procedure is repeated until the fluids are at the proper levels. (It may be necessary to add more mercury in order to force the air over.) Clamp (7) is then closed and the reservoir returned to its support. The air is now removed from the potash pipette by lowering the mercury reservoir (9) until the mercury in the burette is below the bulb, the stopcock is then given a quarter turn to the left, and the air removed by a further lowering of the reservoir. The air in the oxygen absorber is removed in a similar manner by first turning the stopcock to the right. The levels in each case are set at their marks (18) (17) on the manometer tubes.

The tube (1) is connected with a suction pump and a stream of air is kept bubbling through the water-bath. Before one can use the apparatus, it is necessary to absorb the carbon dioxide and oxygen from the air which fills the capillary tubes between the fluids and the stopcock. This is accomplished by drawing about 9 c.c. of air into the burette. Now make a quarter-turn of the stopcock *to the right*, at the same time slightly raising the mercury reservoir (9) so as to put the air under positive pressure and prevent the possible sucking of pyrogallate into the stopcock and burette. Absorption is brought about by alternately raising and lowering the reservoir (9). This pushes the gas back and forth between the burette and absorber, and brings it constantly in contact with new surfaces of fluid. In doing this, the mercury should not be carried above or below the bulb of the burette. This procedure

\*If the handle is not already marked, this can readily be done by dipping the tip in hot sealing wax. The top of the barrel, or the nearest hole in the barrel is the one to be marked.

is repeated 18 to 20 times. The reservoir is then carefully lowered until the pyrogallate enters the manometer tube. Now one connects the burette with the potash pipette. This is accomplished by making a full half-turn of the stopcock *to the right* at the same time slightly raising the reservoir (9), as before, to prevent sucking the alkali into the stopcock and burette. The air is now washed back and forth several times and returned to the burette with the above precautions. The burette is again put in communication with the oxygen absorber by making a full half-turn of the stopcock *in the opposite direction to the hands of a clock*, taking care to slightly raise the reservoir as before. This procedure is repeated several times until the readings of the burette remain constant.

Preparatory to reading the volume, the pyrogallate is brought to its mark (17) on the manometer; the burette is then connected with the potash pipette by a half-turn of the stopcock *to the right*, with the usual slight raising of the mercury reservoir. The level in the potash manometer is now set by rolling the cord back or forth over the hook; at the same time the volume of the control tube is returned to normal by raising or lowering the thistle-tube (15). In this way changes of volume, due to temperature changes, are overcome by equally increasing or decreasing the pressure exerted on the two volumes of gas by the head of fluid in the thistle-tube. In order to be sure that free communication exists between the burette and the manometers, one momentarily pinches the rubber tubing (at the locations of the figures 11 and 24) and determines that the levels in the manometers balance freely with the mercury. A drop of fluid or grease in the stopcock may interfere with the free transmission of pressure and lead to gross errors in the analysis. These obstructions, if present, can usually be dislodged by repeatedly pinching the tubing (24). The levels being properly set at their marks on the manometer tubes, one switches on the light which is placed at the back of the water-bath and reads the burette to the thousandth part of a cubic centimeter by means of a magnifying glass. To do this accurately, one places the eye so that the graduation mark, below the bottom of the mercury meniscus, and its reflection from the mercury, are directly in line; the location of the top of the meniscus between the hundredths divisions is then estimated in tenths. A small amount of acidulated water inside the burette is necessary to insure that the air is saturated with water vapor. If one flushes the burette with the dilute acid immediately before each test, sufficient moisture remains to bring about this saturation. When not in use, the nitrogen remaining after the last analysis is kept in the oxygen absorber; the stopcock is turned so that the burette communicates with the intake-tube (20); the potash reservoir (15) is lowered; and the clamp (7) is opened so that the fluid drops in the control manometer tube. The apparatus can be left safely in this manner, without the danger of alkali being drawn into the burettes as a result of temperature changes.

*Analysis.*—The first step is to start the pump which applies suction (4) and keeps the water well mixed in the bath. The potash reservoir (15) is raised until the surface of its contained fluid is on a level with the mark (18) on the manometer. The reservoir (6) of the control burette is now lowered,

causing the potash to rise in the control manometer to the mark (19); the clamp (7) is then closed tightly and the reservoir returned to its support.

The glass elbow (23) is now attached to the intake tube (20) and the burette flushed out with acidulated water, as previously described. The elbow is removed and the next step is to place the sampling-bottle upon its shelf and to connect the spout closely to the intake-tube, seeing that the stopcock is turned so as to cause these parts to communicate with the curved tube of the bottle. Next it is necessary to remove the nitrogen from the oxygen absorber and to set the levels in the manometer tubes; this is done by raising the mercury reservoir (9) until the small amount of acidulated water in the burette has entered the stopcock; the stopcock is given a quarter turn to the right and the reservoir lowered until the meniscus is at the mark (17); the stopcock is then given a *half-turn to the right*, at the same time slightly raising the mercury reservoir, thus throwing the burette into communication with the potash absorber. The levels in the manometers are set (18) (19) and the burette is connected with the intake-tube by making a quarter turn of the stopcock *to the right*. The burette and intake-tube are completely emptied of air by raising the mercury reservoir (9); this causes the mercury to rise in the burette, forcing the air and most of the acidulated water before it. When the mercury has passed into the curved tube as shown in *E* (Fig. 8), the stopcock of the bottle is given a half-turn, the reservoir lowered, and the expired air passes into the burette as shown in *F* (Fig. 8). (In this manipulation, the reservoir is grasped in the hand and raised slightly forward from the hook, leaving the cord in position). The sample is washed back into the bottle and again drawn into the burette by raising and lowering the mercury reservoir. This air is considerably compressed, and in order to take a sample which can be measured at atmospheric pressure, one sets the mercury meniscus at about 9.2 c.e., the stopcock of the bottle is then given a quarter of a turn, and the reservoir lowered until the burette reads in the neighborhood of 9.85 c.e. Next, the four-way stopcock is carefully given a quarter-turn *to the left*, and the levels in the manometer tubes (18) (19) rapidly reset by manipulating the thistle-tube (15) and the mercury reservoir (9). The volume of gas in the burette is now read to a thousandth part of a c.e. in the manner already described.

The carbon dioxide is absorbed by alternately raising and lowering the mercury reservoir, not permitting the mercury to rise above, or pass below, the bulb of the burette. After passing the gas, in this manner, eight or nine times one resets the levels (18 and 19) and reads the burette. The gas is again passed five or six times and the volume recorded. If the volumes do not agree within 0.002 c.e., the procedure is repeated until check readings are obtained.

To absorb the oxygen, one gently lowers the mercury reservoir (9) until the potash is within an inch of the bend above the mark (18), the stopcock (2) is then given a half-turn *in the opposite direction to the hands of a clock*, at the same time slightly raising the mercury reservoir to avoid the danger of sucking pyrogallate into the stopcock and burette. The gas is passed about



eighteen times into the oxygen absorber. It is then passed twice into the potash pipette, in order to wash out the oxygen which was left in the capillary tube, and again passed into the oxygen absorber an additional nine or ten times. This combined procedure is repeated twice, the pyrogallate meniscus reset (17), the burette thrown into communication with the potash pipette, the levels (18 and 19) readjusted, and the volume read on the burette. Absorption should be continued until check readings are obtained.

These three volumes are now corrected according to the recalibration of the burette. The difference between the second and first readings represents the volume of carbon dioxide in the sample; and this difference divided by the first reading tells the percentage of this gas in the expired air. In a similar manner, to determine the percentage of oxygen in the expired air, one subtracts the third from the second reading, and divides the difference by the original volume.

*Bank of Gas-analyzers.*—The arrangement of a bank of six gas-analyzers is shown in (Figs. 1 and 9). A stout shelf, 8 feet long and 12 inches wide,

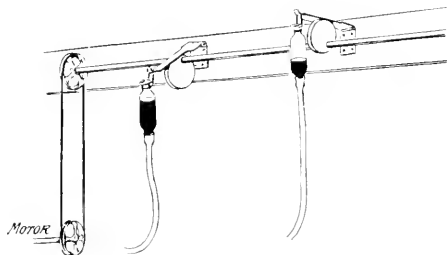


Fig. 12.

is firmly fastened to the wall at a height of 3 feet from the floor. This shelf supports the apparatus, which are placed with intervals of 4 inches between the bases. Beneath the shelf is a water-motor, belted to a speed-reducing gear (1 to 68), which in turn is belted to a countershaft (Fig. 12), supported from the wall at a height of 25 inches above the shelf. This countershaft carries a series of eccentrics which raise and lower the mechanical arms. A  $\frac{3}{4}$  inch iron vacuum pipe, fitted with 6 pet-cocks, is placed along the wall, 2 inches above the shelf. From each of these pet-cocks, tubing leads to the suction-tube (1, Fig. 10) of the apparatus. Suction is applied to the vacuum pipe by means of a water suction-pump. Behind each apparatus is an electric plug to connect with the burette-reading lamp.

With this arrangement, one assistant can readily attend six analyzers. In using the mechanical arms to manipulate the gas, one grasps the mercury reservoir (9) in the right hand, the left hand detaches the cord from the reservoir and places the weight on the shelf. The reservoir is then hooked to the mechanical arm (25, Fig. 10). The arm has a movement of about 4 inches. A rate of 8 or 9 times a minute keeps the mercury moving from the bottom

## RESPIRATION LABORATORY

Case No. <u>9569</u>	Department of the Laboratory	Date <u>11-11-21</u>
Lab No. <u>27110</u>	New York Post-Graduate Medical School	Cause No. <u>1</u>
Name <u>Rose Fraser, age 22</u>	And Hospital	Comp. Bottle No. <u>4, 5 &amp; 6</u>

---

Pneumeter, <u>762.6</u> mm	Log Fact Gasom. .... <u>97313</u>
Temp Gasom. .... <u>23</u> °C.	Log Gasom diff. .... <u>95231</u>
	Log Fact S T F D. .... <u>9523</u>
	Log Total vent. .... (add) <u>87774</u>
	Log Time. .... <u>02117</u>
	Log Vent. per min. (sub) <u>85655</u> = <u>7.19</u> L
	Log % O <sub>2</sub> absorbed. .... <u>63548</u>
	Log O <sub>2</sub> Absorbed (add) <u>49203</u> = <u>310.5</u> cc
	Log Cal value O <sub>2</sub> +log .... <u>4551</u>
	Log Total Cal. per hr. (add) <u>94713</u> = <u>88.54</u> Cal
	Log Surface area. .... <u>15229</u>
	Log Cal. per sq m. hr. (sub) <u>77484</u> = <u>62.35</u> Cal

End. <u>89.9</u> cm	cm
Gas m. Start. <u>0.3</u> cm	m.
Diff. <u>89.6</u> (sub.)	cm.

Distance. <u>10</u> m	<u>30</u> s. = <u>10.5</u> min.
-----------------------	---------------------------------

O <sub>2</sub> Inspired. .... <u>3.31</u> %	
O <sub>2</sub> Expired. .... <u>0.04</u> %	
CO <sub>2</sub> Produced. .... (sub) <u>3.27</u> %	

O <sub>2</sub> Inspired. .... <u>21.15</u> %	
O <sub>2</sub> Expired. .... <u>16.83</u> %	
O <sub>2</sub> Absorbed. .... (sub) <u>4.32</u> %	

Log % O <sub>2</sub> produced. .... <u>51455</u>	
Log % O <sub>2</sub> absorbed. .... <u>63548</u>	
Log Resp Quot. .... (fact) <u>87907</u> = <u>0.76</u>	

Cal. per sq m. hr. (above net) <u>62.35</u> .	
Cal. per sq m. hr. normal <u>37</u> .	
Cal. per sq m. hr. below normal	
Difference. .... (sub) <u>25.35</u>	

Log difference. .... <u>4039</u>	
Log normal. .... <u>5682</u>	
Log B.M. % .... (sub) <u>8357</u>	
BASAL METABOLISM <u>+68</u> %	

Analysis by <u>JSJ</u>	Haldane No. <u>4</u>	Analysis by <u>JSJ</u>	Haldane No. <u>5</u>	Analysis by	Haldane No.
<u>9.780 - 0.02 = 9.778</u>		<u>9.555 + 0 = 9.555</u>			
<u>9.455 - 0.01 = 9.454</u>		<u>9.235 + 0.04 = 9.239</u>			
<u>.324</u>		<u>.316</u>			
<u>7.810 + 0.01 = 7.811</u>		<u>7.625 + 0.03 = 7.628</u>			
<u>1.643</u>		<u>1.611</u>			

Log CO <sub>2</sub> diff. <u>51053</u>	Log O <sub>2</sub> <u>21564</u>	Log CO <sub>2</sub> diff. <u>49969</u>	Log O <sub>2</sub> diff. <u>20710</u>	Log CO <sub>2</sub> diff. ....	Log O <sub>2</sub> diff. ....
Log (orig) <u>97023</u>	Log (orig) <u>97043</u>	Log (orig) <u>98023</u>	Log (orig) <u>98023</u>	Log (orig) ....	Log (orig) ....
Log CO <sub>2</sub> % <u>51050</u>	Log O <sub>2</sub> % <u>22539</u>	Log CO <sub>2</sub> % <u>51746</u>	Log O <sub>2</sub> % <u>22687</u>	Log CO <sub>2</sub> % ....	Log O <sub>2</sub> % ....
CO <sub>2</sub> % <u>3.31</u>	O <sub>2</sub> % <u>16.8</u>	CO <sub>2</sub> % <u>3.30</u>	O <sub>2</sub> % <u>16.86</u>	CO <sub>2</sub> % ....	O <sub>2</sub> % ....

Notes	
<u>Ht - 153 cm</u>	
<u>Wt - 104 lbs</u>	
<u>Surface Area - 1.42 Sq. m.</u>	
<u>Resp - 22</u>	
<u>Pulse - 124, 120</u>	

Avg CO <sub>2</sub> <u>3.31</u> %	Readings by
Avg O <sub>2</sub> <u>16.83</u> %	Checked by
CO <sub>2</sub> +O <sub>2</sub> <u>20.14</u> %	Calculations by
	First check by
	Second check by

FIG. 1

to the top of the burette bulb. The throw of the mercury is regulated by the speed, and by the amount of mercury in the reservoir.

## CALCULATION OF THE BASAL METABOLISM

In calculating the basal metabolism, the scheme worked out by Boothby and Sandiford\* has been adopted. In their calculations these authors use four place logs without the characteristics, and by arranging the various steps

\* For a description of the calculation, and the use of the log, reference should be made to the book Boothby and Sandiford, "The Calculation of the Basal Metabolism," published in this publication.

in their order of sequence, the entire calculation is made on one form-sheet (Fig. 13). This form has bracketed instructions for each step so that the danger of mathematical errors is largely eliminated. For expressing the volume of expired air at normal temperature and pressure, they have devised log tables of the factors which, at one step, make corrections for the brass-scale barometer, water vapor, temperature, and pressure. The triplicate analyses of the expired air are recorded on the same form sheet. Tables are given for computing the volume of oxygen inspired, based on a comparison of atmospheric and expired nitrogen. The oxygen absorbed is determined by subtracting the expired oxygen from the estimated inspired oxygen. One subtracts the carbon dioxide in atmospheric air (0.04 per cent) from that found in the expired sample, in this way determining the carbon dioxide produced. The ratio of carbon dioxide produced to oxygen absorbed is the respiratory quotient, and this value being known, by consulting the tables one can determine the number of calories produced as a result of the consumption of the determined amount of oxygen per hour. This heat production is expressed in terms of calories per square meter of body surface per hour,—the surface area being derived from DuBois height-weight chart. The basal metabolism is recorded as the per cent above or below the average normal standard of Aub and DuBois.<sup>11</sup>

#### SUMMARY

A detailed description has been given of apparatus<sup>\*</sup> used in determining the respiratory exchange in man. The arrangement described is suitable for routine laboratory or institutional use in determining the basal metabolism. The particular features are the use of the full-sized gas mask, the special arrangement of rubber flutter-valves, a newly designed gasometer, the use of a new type of gas-sampling bottle in conjunction with the Henderson-Haldane gas-analysis apparatus, a detailed description of the construction and use of the latter, with several added mechanical features which greatly lessen the labor of gas analyses.

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<sup>\*</sup>This equipment, complete or in part, may be obtained from the C. M. Sorensen Co., 177 East 87th Street, New York.

## DISTRIBUTION OF URIC ACID IN THE BLOOD\*

BY RUTH C. THEIS AND STANLEY R. BENEDICT, NEW YORK CITY

FOR the past two years as opportunity offered, we have been studying the distribution of uric acid between plasma and corpuscles, as well as the permeability of corpuscles to added uric acid. In the meantime Bornstein and Griesbach<sup>1</sup> published a paper dealing with the uric acid content of whole blood and plasma before and after incubation and after heating in a water-bath to 56° for one-half hour. By determining uric acid in the whole blood and serum and determining the corpuscle volume of the blood, they calculated the corpuscle uric acid on a basis of milligrams of uric acid per 100 c.c. of corpuscles. In their study of 20 cases they found 2 in which serum and corpuscle uric acid were equal on a volume basis; 13 in which serum uric acid was less than corpuscle uric acid; and 5 in which serum uric acid was greater than the uric acid in an equal volume of corpuscles.

We employed the Benedict<sup>2</sup> modification of the Folin-Denis method for uric acid determinations, using 10 c.c. of blood, and aluminium cream for the second protein precipitation instead of colloidal iron. Corpuscle volume was determined by the hematocrit. For the bloods from the wards of the Memorial Hospital we are indebted to Dr. William Stone; for bloods from the Roosevelt Hospital to Dr. William Lyle. The former bloods were defibrinated and the latter oxalated.

In a series of 104 cases we have determined sometimes both plasma and corpuscle uric acid, sometimes calculated corpuscle uric acid from the known corpuscle volume and sometimes calculated from the average corpuscle volume of 35 per cent. Finally the permeability of the corpuscles to added uric acid

TABLE I  
DISTRIBUTION OF URIC ACID BETWEEN PLASMA AND CORPUSCLES

CASE NO.	WHOLE BLOOD URIC ACID MG. PER 100	SERUM URIC ACID MG. PER 100	CORPUSCLE URIC ACID MG. PER 100
1	3.1	3.1	3.1
2	2.8	3.0	1.8
3	3.6	3.4	3.7
4	2.6	3.0	1.8
5	6.0	6.4	2.3
6	2.7	2.6	2.8
7	1.8	1.3	1.7
8	2.9	2.6	2.6
9	3.7	2.6	3.8
10	3.1	3.5	2.6
11	3.7	3.1	3.3
12	3.9	2.3	3.1

\*Fellow of the Huntington Fund for Cancer Research, Memorial Hospital, and the Harriman Research Laboratories, Roosevelt Hospital, New York.

was tested. Both defibrinated and oxalated bloods were used. From the figures which follow we can see that the various methods of approach serve to show that the relationship is a fairly constant one. Cases are divided almost equally between those in which uric acid is equally divided or those in which it is greater in the plasma.

The 12 cases shown in Table I represent those in which uric acid was determined on the corpuscles as well as whole blood and serum. Corpuscles were washed twice with .8 per cent NaCl. Fifty per cent of those bloods show an equal distribution with one exception; the others show that uric acid is higher (.9-4.1 mg. per 100) in the serum than in an equal volume of corpuscles.

TABLE II

## DISTRIBUTION OF URIC ACID BETWEEN PLASMA AND CORPUSCLES\*

Based on determinations in plasma and whole blood, and calculation from corpuscle volume.

CASE NO.	DIAGNOSIS	WHOLE BLOOD URIC ACID MG. PER 100	PLASMA URIC ACID MG. PER 100	CORPUS-CLE URIC ACID MG. PER 100	CORPUS-CLE VOLUME %
12	Neurasthenia	3.2	4.9	-	34
13	"	1.7	3.2	-	30
14	"	3.4	4.2	2.6	46
15	Normal (?)	4.2	5.6	1.0	31
16	"	1.8	2.6	-	26
17	Pregnancy	3.4	4.3	1.7	36
18	"	4.4	5.5	3.2	46
19	"	2.2	3.5	-	41
20	"	3.5	5.5	-	33
21	Previous eclampsia	3.3	4.0	1.9	35
22	Ca. Uterus	3.6	4.8	-	17
23	Brain Tumor	4.7	6.7	-	31
24	Ch. Nephritis	5.8	6.9	1.3	21
25	Cardio. Neph:	2.8	2.1	4.6	28
26	Pregnancy	2.1	2.2	-	26
27	"	3.6	3.3	-	34
28	"	2.3	2.4	-	37
29	Normal	2.3	1.9	-	38
30	Syphilis	3.5	5.8	-	26
31	Arterio Sclerosis	1.9	1.9	-	28
32	Nephritis Decomp.	2.8	3.0	-	27

\*Where the variation between the uric acid content of whole blood and of plasma does not exceed .5 mg. per 100 c.c. it is assumed that the uric acid content of plasma and corpuscles is equal for an equal volume.

Table II shows a series of 22 cases of oxalated bloods with corpuscle uric acid determined from the known corpuscle volume. Since a small error in reading of either whole blood or plasma uric acid would cause a very great difference in the calculated corpuscle uric acid, plasma uric acid that was 0.5 mg. per 100 higher or lower than the whole blood was considered equal. The corpuscle would then be the same also. These results may be summarized as follows: 8, or 36 per cent, give equal values; in 13, or 59 per cent, plasma uric acid is greater than corpuscle uric acid, with 7 negative in the corpuscles and 1 in which plasma uric acid is less than corpuscle uric acid.

Thirty oxalated bloods, where corpuscle volume was arbitrarily set at 35 per cent (average of corpuscle volume in Table I) show that 15, or 50 per cent, have equal values. Ten, or 33 per cent, have plasma uric acid greater than corpuscle uric acid, and 5, or 16 per cent, have plasma uric acid less than corpuscle uric acid. This is the only series in which there is such a large percentage of cases in which the corpuscle uric acid exceeds the plasma uric acid. Twenty cases of defibrinated bloods show 10, or 50 per cent, equal values; 9, or 45 per cent, with plasma uric acid greater than corpuscle uric acid, and 1 with plasma uric acid less than corpuscle uric acid.

Since each series showed practically the same relationship between plasma and corpuscles, we were interested to see whether the corpuscles are permeable to added uric acid. Uric acid was dissolved in lithium carbonate solution and was added to the blood in quantities of from 2 to 5 mg. per 100 c.c. of blood and left overnight. Whole blood uric acid, plasma uric acid before and after addition of uric acid and corpuscle volume were determined. Table III shows

TABLE III  
PERMEABILITY OF CORPUSCLES TO ADDED URIC ACID\*

CASE NO.	WHOLE BLOOD URIC ACID MG. PER 100	PLASMA URIC ACID MG. PER 100	CORPUSCLES URIC ACID MG. PER 100	CORPUSCLE VOLUME %	DISTRIBUTION OF ADDED URIC ACID
34	3.3	4.7	1.1	32	Plasma
35	2.5	3.7	—	32	"
36	1.8	2.5	—	26	"
37	1.7	2.4	—	27	"
38	2.4	4.0	—	30	Equally distributed
39	1.7	3.7	—	35	"
40	2.8	3.4	1.3	30	Plasma
41	2.7	4.6	—	40	Equally distributed
42	3.5	3.0	—	30	Plasma
43	4.2	3.7	—	30	"
44	2.4	2.6	—	28	Equally distributed
45	1.3	1.2	—	23	Plasma
46	2.2	2.5	—	34	"
47	1.5	1.8	—	26	"
48	2.6	3.1	—	29	"
49	3.9	4.4	—	34	Equally distributed
50	1.2	1.3	—	39	Plasma
51	.8	.8	—	32	"
52	5.3	5.5	—	36	"
53	1.7	2.0	—	33	Equally distributed

\*Where the variation between the uric acid content of whole blood and of plasma does not exceed .1 mg. per 100 c.c. it is assumed that the uric acid content of plasma and corpuscles is equal for an equal volume.

the results of these determinations, the last column showing whether added uric acid was concentrated in the plasma or equally distributed. Sixty per cent of the 20 cases show equal values in the original blood and in 40 per cent plasma uric acid is greater than corpuscle uric acid, and 6 of these show no uric acid in the corpuscles. In 14 cases the added uric acid is all present in the plasma; 5 of these had shown the plasma of the original blood higher than the corpuscles, while in 9 the uric acid had been equally distributed. In 6 cases the added uric acid was equally distributed; in 3 of these the original uric acid had been equally distributed.

If we consider whole blood and plasma uric acids equal if there is a difference of .5 mg. between them in Bornstein and Griesbach's table as we did in our calculation, we find that 35 per cent of their cases have equal values; in 45 per cent serum uric acid is greater than corpuscle uric acid; and in only 20 per cent is serum uric acid greater than corpuscle uric acid. In our series of 104 bloods only 8 have a higher corpuscle uric acid, while all other bloods show either equal distribution or higher concentration in the plasma.

This holds whether the bloods are defibrinated and obtained from patients suffering from cancer and allied diseases (Memorial Hospital) or oxalated and obtained from a general hospital service (Roosevelt Hospital). As a matter of fact, out of the 42 cases in which corpuscle volume was determined by the hematocrit there were 13 in which there was no uric acid in the corpuscles. When uric acid is added to the blood, the plasma contains all the added uric acid in a large percentage of cases.

#### SUMMARY AND DISCUSSION

1. Uric acid was determined in plasma and corpuscles in 104 cases, 51 of which showed equal distribution; 45 showed plasma uric acid greater than corpuscle uric acid and 8 showed a greater amount of uric acid in the corpuscles than the plasma.

2. This relationship holds whether the blood is oxalated or defibrinated and does not depend on the pathologic condition.

3. Added uric acid did not penetrate the corpuscles in 70 per cent of 20 bloods studied. In 30 per cent of the cases the added uric acid was equally distributed between corpuscles and plasma.

The marked difference in permeability of the corpuscles of certain bloods for added uric acid is of interest, and suggests that other cells in the body may show similar differences in permeability. Such findings may tend to throw light on the questions involved in specific uric acid retention in the organism.

#### REFERENCES

- <sup>1</sup>Bornstein and Griesbach: *Biochem. Ztschr.*, 1919, ci, 184.  
<sup>2</sup>Benedict: *Jour. Biol. Chem.*, 1915, xx, No. 4.

## VENTILATION, WEATHER, AND THE COMMON COLD\*

### A STUDY OF THE PREVALENCE OF RESPIRATORY AFFECTIONS AMONG SCHOOL CHILDREN AND THEIR ASSOCIATION WITH SCHOOL VENTILATION AND THE SEASONAL CHANGES IN WEATHER

*(Continued from page 510)*

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#### ANALYSIS OF SICKNESS RATES IN INDIVIDUAL ROOMS AND SCHOOLS

AS we have pointed out in the introductory remarks, great care must be taken in drawing conclusions as to the correlation of different facts from the average results of a group. In the study before us it will be necessary to inquire into the records of each school and of the individual rooms to see whether they agree uniformly with the characteristics of the group.

In Tables XI and XII are assembled records for each room covering the nature of air conditions and the amount of respiratory sickness.

It is noticeable that there is a wide variation in respiratory illness. Room 415 (Type B) at P. S. 22 in the second study had no absences whatever from respiratory illness. Room 311 (Type C), P. S. 115, in the second study, has a rate of 50.4. These represent the minimum and maximum limits. The range of respiratory sickness-in-attendance rates is even greater—from zero to 316.

Room 311, P. S. 59 (Type A) is the most congested in the first study, there being but 6.5 square feet of floor space per pupil. In spite of this crowded condition, the absence rate from respiratory disease is only 0.6—one of the lowest. On the other hand, Room 202, P. S. 165, being the least congested, with 19.6 square feet per pupil, has a respiratory absence rate of 37.0, a very high figure. Overcrowding does not inevitably lead to respiratory illness.

The average temperature of Type A rooms was about 59 degrees in both studies. This is much colder than the ordinary school room. In fact, it seems from our general experience entirely too cold for public school children, and yet, on looking over the absence rates, there is no indication that these children had more colds as a result. In fact, the average absence rate for the entire group is lower than the other two ventilation types, as has already been pointed out. In the first study there are only two rooms in Type A with rates over 20. There are three each in Types B and C. Respiratory sickness among those present in school is greater in Type A than in B, but less in A than in C.

In spite of the well intentioned efforts to balance the three types of rooms in the matter of schools and type of pupil, this could not be carried out to



TABLE XI  
RECORDS OF INDIVIDUAL ROOMS IN FIRST STUDY

SCHOOL	ROOM	RESPIRATORY SICKNESS RATES			PERCENT SESSIONS			PERCENT PRESENT			GRADE	SQ. FT. OF FLOOR SPACE PER PUPIL
		AMONG ABSENTEES	AMONG THOSE IN ATTENDANCE	TOTAL	AV. PREVA.	AV. REL. HUMIDITY	EST. PRESENT	ODDROOMS	GIRLS	GRADE		
Type A—Cold Open Window Rooms												
12	203	15	9.6	24.6	61.7	45	87	1	49	2A	15.5	
	109	8.6	3.1	11.7	59.5	45	95	0	100	4B	13.5	
	110	5.0	16.0	21.0	60.3	43	81	0	100	1B	13.8	
	202	11	0	11.0	56.9	52	96	1	0	3A	18.9	
	203	11	.6	11.6	57.4	46	96	1	0	4A	13.6	
20	201	11	0	11.0	57.5	50	92	1	0	3B	15.1	
	205	21	2.1	23.4	56.8	47	93	0	0	2B	15.0	
	206	17	1.8	18.8	57.5	48	91	3	0	5A	11.4	
	207	11	1.7	12.7	56.9	45	96	1	0	1B	11.7	
	211	.6	21.0	21.6	59.0	58	28	9	27	3B	6.5	
59	213	8.2	210	218.2	57.7	58	86	13	5	2B	9.9	
	412	5.8	91.0	96.8	58.6	57	27	72	98	4A	6.9	
	413	8.5	72.0	80.5	61.1	57	5	8	73	4B	7.8	
	414	.6	71.0	71.6	57.1	57	50	6	60	4A	7.8	
	301	6.1	33.5	39.9	57.8	47	39	27	47	5A	9.1	
73	309	10.8	42.4	53.2	56.3	41	51	21	46	3A	8.4	
	402	27.8	72.0	97.8	62.2	45	17	71	57	6A	9.9	
	407	11.5	21.6	33.1	61.0	46	5	88	67	5B	9.2	
Type B—Moderate Temperature, Open Window Rooms												
18	304	3.1	55.7	58.8	70.7	25	25	8	0	4B	12.5	
	302	31	38.1	69.1	70.7	25	16	1	18	3B	11.9	
	316	16.4	16.5	62.9	69.7	21	29	9	100	5A	12.3	
	405	1.5	14.8	19.3	66.5	43	36	5	35	3B	13.6	
	108	11.3	13.5	24.8	67.5	42	41	0	100	1B	13.1	
22	111	5.1	17.0	22.0	65.9	41	40	1	100	1A	13.8	
	115	2.7	7.4	11.1	69.9	45	9	8	41	5B	8.1	
	116	8.5	6.5	15.0	69.7	48	5	30	23	4B	7.9	
	418	8.9	0	8.9	69.3	43	13	3	0	5A	7.8	
	302	6.9	0	6.9	62.2	40	80	1	0	3B	14.9	

TABLE XI (CONTINUED)

NO. ROOM	RESPIRATORY SICKNESS RATES			PER CENT SESSIONS			GRADE	SQ. FT. OF FLOOR SPACE PER FUTH.
	AMONG ABSENTEES	AMONG THOSE IN ATTENDANCE	TOTAL	AV. TEMP.	AV. REL. HUMIDITY	ESP. FRESH AIR		
29	21	46	21.6	60.2	45	88	1	3A
	14	0	1.4	62.3	45	65	4	4A
	15.1	60.9	76.0	64.8	56	5	6	2B
	10.9	90.6	101.5	65.7	32	12	13	5A
	1.5	14.6	16.1	65.6	32	4	12	4A
73	32	1.1	4.3	66.8	46	0	41	1A
	10.2	1.2	11.4	62.8	43	13	35	3B
	10.1	23.9	34.3	66.4	47	0	56	5A
	10.4	21.3	31.7	69.4	30	21	3	5B
	5.7	37.9	43.6	67.7	33	26	3	4B
165	26.1	47.5	73.9	71.8	27	6	21	11.1
2	13.0	64.2	77.2	69.5	30	26	18	3A
	8	32.7	33.5	68.6	25	39	15	4A
	13.3	47.2	60.5	69.2	24	64	6	5A
	7.0	4.1	11.1	68.4	42	11	3	4B
	10.5	12.0	22.5	67.4	46	14	1	4B
59	8.9	25.4	34.3	68.5	43	14	3	1A
	16.9	188	204.9	68.5	48	23	15	100
	17.9	149	166.9	68.9	47	19	23	100
	16.8	140	156.8	67.8	49	21	10	100
	10.1	163	173.4	67.8	50	19	24	100
147	8.0	96.6	104.6	68.3	50	47	3	5B
	12.6	97.3	109.9	68.0	50	37	3	100
	8.1	45.8	53.2	66.4	38	3	28	3A
	1.5	72.5	74.0	68.0	36	9	47	4B
	9.6	21.5	31.1	67.5	36	8	26	5A
165	31.0	33.6	67.6	70.2	30	3	9	4A
	27.0	81.8	118.8	72.2	24	0	10	3B
	35.2	156.0	191.2	70.8	24	4	6	4A
	12.0	33.6	45.6	70.5	27	11	22	5B
								12.0

Type C—*Moderate Temperature, Poor Ventilation, Closed Window Rooms*

TABLE XII  
RECORDS OF INDIVIDUAL ROOMS IN SECOND STUDY

RESPIRATORY SICKNESS RATES							PER CENT		
SCHOOL	ROOM	AMONG ABSENTEES	AMONG THOSE IN ATTENDANCE	TOTAL	AV. TEMP.	AV. REL. HUMIDITY	ESP. FEELS	CHORUS	GRADE
Type A—Cold Open Window Rooms									
12	303	3.9	46.6	50.7	59.9	58.0	28	0	2 A
	409	8.1	13.7	21.8	61.3	54.4	18	0	4 B
	410	11.1	14.2	25.3	60.3	56.0	33	0	4 B
	202	6.9	65.0	71.9	58.7	41.3	65	4	3 A
39	203	13	40.0	53.0	56.2	43.4	72	2	4 A
	204	21.6	45.9	67.5	56.6	43.1	72	1	3 B
	205	6.7	30.3	37.0	58.7	42.2	46	7	3 B
	206	9.3	24.9	34.2	58.3	40.3	55	0	3 A
59	207	11.7	18.4	30.1	57.6	41.4	60	2	4 B
	308	4.0	165	169	60.0	48.0	100	0	3 B
	311	8.5	144	152.5	58.5	48.9	100	0	3 A
	313	26.8	213	239.8	59.0	44.7	85	11	2 A
	412	4.3	150	154.3	58.1	45.1	100	0	4 A
	413	9.2	147	156.2	60.0	48.0	98	2	4 B
	414	3.0	124	127	57.8	48.4	100	0	4 A
	416	8.0	91.3	99.3	57.6	46.5	100	0	4 B
73	301	5.1	44.1	49.2	60.2	44.8	71	2	5 B
	309	11.6	34.8	46.4	58.9	45.9	67	2	4 A
	402	6.1	18.4	24.5	60.5	44.7	72	5	6 A
	403	6.2	39.4	45.6	63.5	45.3	50	29	5 B
Type B—Moderate Temperature, Open Window Rooms									
2 Bx.	301	10.7	9.7	20.4	68.6	38.5	0	13	2 B
	302	7.7	5.8	13.5	69.0	38.5	0	23	2 B
	316	11.6	12.2	23.8	65.4	40.0	0	24	5 A
	405	9.5	33.2	42.7	62.2	57.8	6	1	4 A
12	408	15.9	31.4	47.3	63.5	59.0	1	2	3 B
	411	9.9	5.8	15.7	63.8	60.6	3	10	4 A
	415	0	0	0	67.6	57.7	17	82	5 B
	416	5.5	2.7	8.2	68.2	56.0	23	72	6 A
	418	.9	24.6	25.5	67.3	56.7	29	65	5 B
33 Bx.	13	21.4	55.7	77.1	69.3	31.3	0	9	8 B
	23	27.5	47.5	75.0	69.4	37.2	1	21	7 A
	302	10.9	33.6	44.5	63.0	40.3	19	0	4 A
	308	8.6	42.8	51.4	65.0	37.7	23	3	5 A
39	408	2.9	58.0	60.9	65.2	39.0	9	31	5 A
	318	3.7	187	190.7	64.9	48.5	4	61	3 A
	415	16.2	158	174.2	58.8	48.2	100	0	4 B
	305	9.7	23	32.7	61.6	46.5	45	10	4 B
73	406	2.7	41	43.7	67.1	43.3	17	35	3 A
	408	16	51	67	61.1	43.6	74	4	3 B
	302	6.4	37	43	67.0	38.2	17	62	5 B
	313	7.1	7.0	14.1	67.3	41.8	71	17	4 A
97	501	3.0	55	58	65.9	44.8	59	18	6 B
	312	23.3	—	—	67.8	28.8	3	3	4 B
	502	40.2	—	—	67.9	26.2	2	3	5 B
	115								

TABLE XII (CONTINUED)

SCHOOL	RESPIRATORY SICKNESS RATES				PER CENT SESSIONS				
	ROOM	AMONG ABSENTEES	AMONG THOSE IN ATTENDANCE	TOTAL	AV. TEMP.	AV. REL. HUMIDITY	ESP. FRESH	ODOROUS	GRADE
165	503	29.8	—	—	67.0	29.5	0	3	5 B
	302	1.3	99	100.3	67.2	35.9	0	0	5 B
	306	10.4	56	66.4	66.8	35.7	0	0	4 B
	309	8.1	33	41.1	68.9	36.1	0	0	4 B
<i>Type C—Moderate Temp., Fan Ventilated, Closed Window Rooms</i>									
2 Bx.	207	22.1	25.5	47.9	66.1	39.6	0	13	2 A
	307	13.2	17.6	30.2	65.8	41.2	0	35	3 B
	310	1.3	3.8	5.1	66.5	41.5	0	20	4 B
	402	4.8	5.6	10.4	66.5	57.0	75	16	6 A
22	403	3.5	1.3	4.8	65.8	56.2	77	23	4 B
	404	11.4	18	32.1	66.5	54.9	66	17	4 B
	14	29.3	23	52.3	71.0	28.6	1	2	6 B
	21	45.4	125	170.4	70.2	32.0	3	3	5 A
51 Bx.	203 (Hum.)	4.0	118	152	67.6	43.6	0	39	6 A
	205	14.7	50	64.7	67.4	29.0	0	2	6 A
59	203	16.8	182	198.8	69.2	47	10	12	6 B
	205	13.9	275	288.9	69.2	45.5	17	5	6 B
	206	9.9	316	325.9	69.6	46	16	1	6 A
	501	12.4	256	268.4	68.9	49	7	6	5 B
97	503	9.1	197	206.1	69.2	47.9	25	1	5 B
	505	8.3	112	120.5	68.7	49.3	2	13	6 A
	303	2.3	34.1	36.4	68.0	43.1	84	2	4 B
	308	2.7	34.7	37.4	68.3	40.2	79	4	5 A
	502	6.8	13.3	50.1	66.3	44.8	91	3	6 B
115	308	14	24.8	68.8	67	27.2	2	5	4 A
	311	50.4	51.6	102	68.2	25.2	0	6	4 B
117	210	16.6	11.9	28.5	68.1	42.4	30	13	3 A
	304	3.6	6.8	10.4	67.7	40.1	22	36	4 B
	305	4.2	12.4	16.6	67.1	42	49	9	5 A
165	201	11.9	196	207.9	68.4	33.3	0	0	4 A
	202	5.3	151	156.3	68.0	34.5	0	0	3 B
	220	10	181	191	69.2	35	0	0	4 A
	308	1.8	154	155.8	68.7	35	0	0	5 B

the degree desired. If the pupils in one district are by reason of hereditary and environmental influences more susceptible to colds, then this school will unduly raise the sickness rate in the ventilation type within which the majority of its records fall.

Of the 12 schools used in the two studies, only one possessed all three examples of ventilation. In one other instance the three types were represented by two schools a block or so apart, one school having Type C and the other, Types A and B. In all other instances there were not more than two types represented within a school building, some buildings having A and B and others, B and C. The division of rooms is revealed in the table below.

One can readily appreciate by looking at the table how the results would be affected if, say, School 39 were given to very little sickness and School 147 to

TABLE XIII  
DISTRIBUTION OF TEST ROOMS AMONG THE TWELVE SCHOOLS BY VENTILATION TYPE

SCHOOL	FIRST STUDY			SECOND STUDY		
	A	B	C	A	B	C
12	3	3	0	3	3	0
147	0	0	3	0	0	3
22	0	3	3	0	1	3
59	5	3	6	7	2	6
73	4	3	0	4	3	0
165	0	3	4	0	3	4
39	6	3	0	6	3	0
2 Bx.	0	3	3	0	3	3
33 Bx.				0	2	2
51 Bx.				0	0	2
97				0	3	3
115				0	3	2
Total	18	21	19	20	28	28

a great deal. In the summary of all rooms Type A, having 6 rooms in School 39 would have a low sickness rate, not because of ventilation, but because of its personnel, and Type C would be inclined to have a high rate, not because of ventilation but because of the numbers of children from School 147. Theoretically this influence should have been eliminated at the beginning, but actually this was found impossible.

The total respiratory illness rate including both absentees and those present in each school is shown in Table XIV.

TABLE XIV  
TOTAL RESPIRATORY ILLNESS RATES BY SCHOOLS

SCHOOL	FIRST STUDY	SECOND STUDY
97	—	46.7
22	100.3	13.6
12 & 147	31.1	27.9
73	37.4	44.2
51 Bx.	—	106.4
59	103.1	193.3
39	12.6	51.6
2 Bx.	55.9	24.2
165	77.9	123.3
33 Bx.	—	92.6

A considerable variation is seen in the illness rates. Schools 59 and 165 are relatively high in both studies. Schools 12, 147 and 73 are low in both. School 22 is high in the first study and extremely low in the second.

We may examine the effect of ventilation apart from these extraneous influences mentioned first by inspecting the records of each school by itself and secondly by balancing the influence which each school exerts on the total.

In an effort to illustrate the comparison of illness rates within each school we have prepared Charts I and II, the former showing Types A and B and the latter, B and C. Both measures of illness among the absent and among those present are included. The frequency with which one ventilation type exceeds the other in amount of illness conveys an impression that is not brought out in the averages for each ventilation type.

In the comparison of the window ventilated rooms from the chart, Type B exceeds Type A in respiratory illness in nine instances. In the remaining seven instances Type A exceeds Type B. There is then no prevailing superiority of one type over another. It will be noted in the summary at the bottom of the chart, where the rates have been averaged, that Type B shows less re-

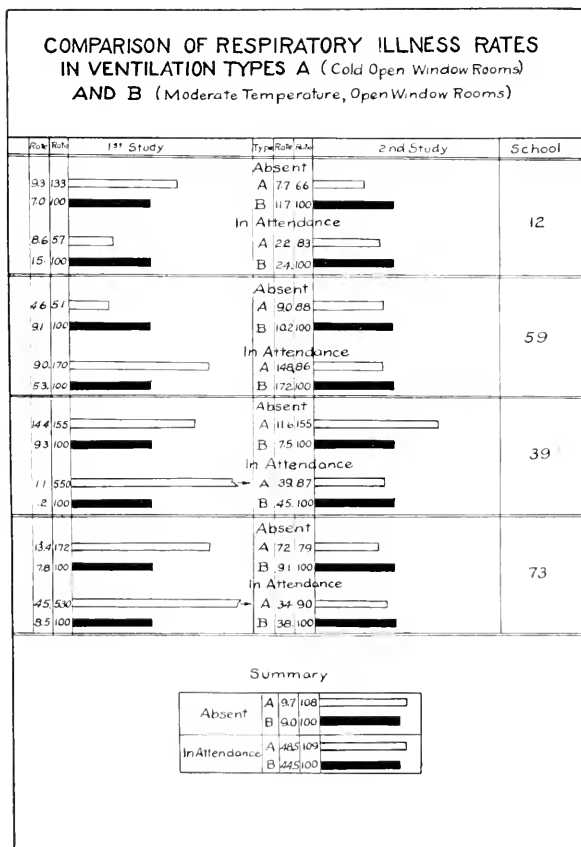


Chart I.

spiratory illness than A. This is due to the influence of several high rates in Type A. Incidentally this illustrates how an erroneous impression may be gained from averages alone.

In the comparison between Types B and C, the latter exceeds the former in respiratory illness in eighteen instances; whereas B exceeds C in only seven

instances. This result is much more significant than in the previous comparison. The averages of the rates are consistent with the tendency of the individual instances. With the new schools used in the second study included, the average for Type C exceeds B in both measures of respiratory illness. The same is true with the new schools omitted.

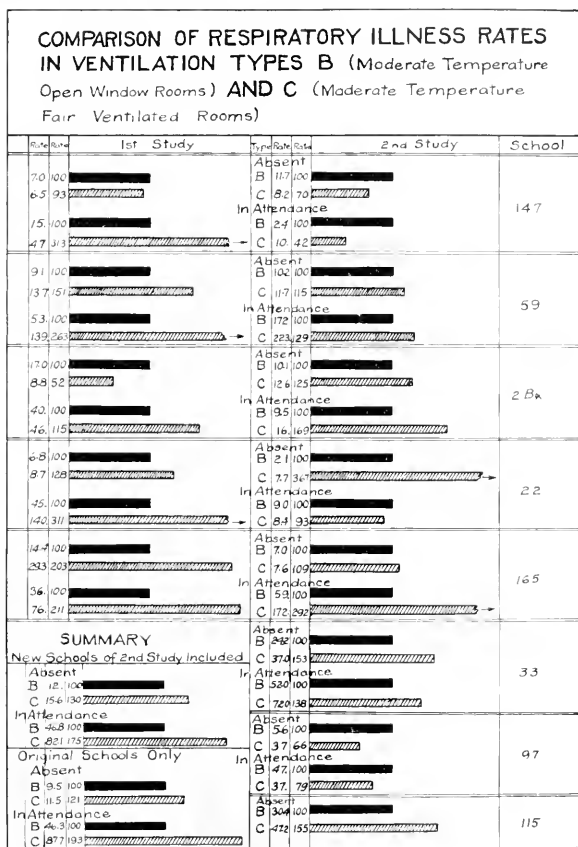


Chart II.

This analysis confirms what has been brought out previously—that the difference between Types A and B is insignificant; whereas, between B and C there is a distinct excess of illness in the fan ventilated rooms.

In School 59 all three types of ventilation are represented. We have in this instance a good measure of ventilation influences on pupils of the same

general characteristics. The fan ventilated rooms have the greatest respiratory illness in both studies. The relative positions of A and B are not the same in the two studies, the cold rooms having more illness in the first study and less in the second.

TABLE XV  
RESPIRATORY ILLNESS RATES IN THREE VENTILATION TYPES AT SCHOOL 59

VENTILATION TYPE	FIRST STUDY			SECOND STUDY		
	SICKNESS CAUSING ABSENCE	SICKNESS IN SCHOOL	SUM	SICKNESS CAUSING ABSENCE	SICKNESS IN SCHOOL	SUM
A	4.6	90	94.6	9.0	148	157
B	9.1	53	62.1	10.2	172	182
C	13.7	139	152.7	11.7	223	235

The room temperature for each type averaged as follows:

A	58.8	58.7
B	65.4	61.9
C	68.2	69.1

The fan ventilated rooms were the warmest, exceeding the Type B rooms by 2.8 degrees in the first study and by 7.2 degrees in the second. These results are consistent in showing less illness in the window ventilated rooms.

In one other instance the three ventilation types are to be found divided between two schools within a block of each other and for all practical purposes the characteristics of the pupils are the same. The sickness rates for Schools 12 and 147 are shown in Table XVI.

TABLE XVI  
RESPIRATORY ILLNESS RATES IN THREE VENTILATION TYPES AT SCHOOLS 12 AND 147

VENTILATION TYPE	FIRST STUDY			SECOND STUDY		
	SICKNESS CAUSING ABSENCE	SICKNESS IN SCHOOL	SUM	SICKNESS CAUSING ABSENCE	SICKNESS IN SCHOOL	SUM
A	9.3	8.6	17.9	7.7	22	29.7
B	7.0	15	22.0	11.7	24	35.7
C	6.5	47	53.5	8.2	10	18

(Temperature—1st Study A 60.5, B 66.6, C 67.3; 2nd Study, A 60.5, B 63.2, C 67.6.)

The fan ventilated rooms appear to better advantage in this instance, for illness is lowest in Type C in the second study. In the first study the total illness is greatest in the fan ventilated rooms, although the illness causing absence is the lowest of the three types.

The Type C rooms at School 59 were unusually well equipped with mechanical ventilation facilities. The air is humidified before entering the room, and the blowers are capably managed. The fan equipment at School 117 is older and the rooms were not thoroughly aerated at all times, and windows were frequently found open.

In view of the oft repeated assertion that humidification and air washing in combination with the plenum fan is from many standpoints a superior



form of ventilation, the figures of this study are of special interest. A modern form of mechanical ventilation with warm temperature is associated with more respiratory sickness than naturally ventilated rooms with gravity exhaust. On the other hand, fan ventilation, lacking many modern features, as in P. S. 147, is associated with less respiratory illness than naturally ventilated rooms. At P. S. 147 the temperature of the fan rooms was lower than at P. S. 59. Raising the temperature over 68 degrees would seem to be more disturbing to health than reducing the volume of air passing through the rooms.

The temperature of the A rooms in all instances was lower than those selected as B rooms. In general the C rooms were warmer than B, although there were some exceptions to this.

TABLE XVII  
AVERAGE TEMPERATURE AND TOTAL RESPIRATORY ILLNESS RATES BY  
VENTILATION TYPES IN EACH SCHOOL

SCHOOL	VENTILATION TYPE	FIRST STUDY		SECOND STUDY	
		TEMP.	RATE	TEMP.	RATE
12	A	60.5	17.9	60.5	29.7
	B	66.6	22.0	63.2	35.7
147	C	67.3	33.5	67.6	18.2
39	A	57.2	15.5	57.7	50.6
	B	61.6	9.5	61.4	52.5
59	A	58.8	94.6	58.7	157
	B	65.4	62.1	61.9	182
	C	68.2	153	69.1	235
73	A	60.1	58.4	60.8	41.2
	B	65.3	16.3	66.6	47.1
2 Bx.	B	70.4	57.0	67.7	19.6
	C	69.1	54.8	66.1	28.6
22	B	69.6	51.8	67.7	11.1
	C	68.1	22.7	66.3	16.1
165	B	69.6	50.4	67.6	66.0
	C	70.9	105	68.6	186
33 Bx.	B	—	—	69.4	76.2
	C	—	—	70.6	169
115	B	—	—	67.6	30.4*
	C	**	—	67.6	47.2*
97	B	—	—	66.7	52.6
	C	—	—	67.6	40.7

\*This is absence illness only.

Whenever temperatures are over 68 degrees, the warmer rooms have the greater sickness, regardless of whether Type B or C. When temperatures are below 68, the window rooms have less sickness in four instances and more sickness in two instances.

It may be pointed out in this connection that even where the temperatures of a fan and window room, as measured at the three-foot level, are identical, the window room is actually cooler, for the temperature at the floor level is always lower in the window rooms.

The second means of shedding light on the significance of the grand averages is by equalizing the influence of each school in each ventilation group. In doing this we have combined the two studies, omitting the schools that were not represented in both. Where there are 9 rooms at a school, 6 in Type A and 3 in Type B, we have reduced the number in the first type to three by averaging the two highest readings, the two lowest and the two intermediate. Where there are four readings, we have averaged the two highest and used the other two as they stand.\*

We have illustrated in Tables XVIII and XIX the manner of making this computation by showing the selected rates used along with the original figures.

In Table XX will be found the averages of the rates both actual and ad-

TABLE XVIII  
ACTUAL AND SELECTED RESPIRATORY ILLNESS RATES BY ROOMS  
COMPARISON OF TYPES A AND B (BOTH STUDIES COMBINED)

SCHOOL	ABSENCE RATE				ILLNESS IN ATTENDANCE RATE			
	TYPE A		TYPE B		TYPE A		TYPE B	
	ACTUAL	SELECTED	ACTUAL	SELECTED	ACTUAL	SELECTED	ACTUAL	SELECTED
12	15.	15.	4.5	4.5	10.	10.	15.	15.
	8.6	8.6	11.3	11.3	3.	3.	14	14.
	5.0	5.0	5.1	5.1	16.	16.	17.	17.
	3.9	3.9	9.5	9.5	47.	47.	33.	33.
	8.1	8.1	15.9	15.9	14.	14.	31.	31.
	11.1	11.1	9.9	9.9	14.	14.	6.	6.
39	11.	11.	6.9	6.9	2.	2.	0.	0.
	11.		21.	21.	2.			
	14.	14.	1.4	1.4	2.	1.	1.	1.
	14				.6			
	21	19.			0	0	0	0
	17				0			
	6.9	6.8	10.9	10.9	65.	55.	34.	34.
	6.7		8.6	8.6	46			
	9.3	10.5	2.9	2.9	40	35.	43.	43.
	11.7				30			
	13.0	17.3			25.	21.	58.	58.
	21.6				18.			
59	.6	.6	15.1	15.1	210.	151.	61.	61.
	.6		10.9	10.9	91			
	8.2	8.4	1.5	1.5	72.	47.	91.	91.
	8.5				21.			
	5.8	5.8			74.	74.	15.	15.
	26.8		3.7	3.7	213.			
	9.2	18.	16.2	16.2	165	189.	187.	187.
	8.5			10.	124			
	8.	8.3			91	108	158.	158.
	4.3				144			
	3.0	3.8			150.	147.		173.
	4.				147.			
73	25.8	18.8	3.2	3.2	57.	57.	1.	1.
	11.5		10.2	10.2	34.	34.	1.	1.
	6.4	6.4	10.4	10.4	32.	32.	24.	24.
	10.8	10.8						
	11.6	8.9	9.7	9.7	42.	42.	23.	23.
	6.2		2.7	2.7	35.	35.	41.	41.
	6.1	6.1	16.	16.	18.	18.	51.	51.
	5.1	5.1						

\*This might be done by averaging the two lowest or the two intermediate rates, letting the highest value stand. The difference, however, is too slight to alter the final result.

TABLE XIX  
ACTUAL AND SELECTED RESPIRATORY ILLNESS RATES BY ROOMS  
COMPARISON OF TYPES B AND C (BOTH STUDIES COMBINED)

SCHOOL	ABSENCE RATES				ILLNESS IN ATTENDANCE RATES			
	TYPE B		TYPE C		TYPE B		TYPE C	
	ACTUAL	SELECTED	ACTUAL	SELECTED	ACTUAL	SELECTED	ACTUAL	SELECTED
2 Px.	3.1	3.1	13.	13.	36.	36.	64.	64.
	31.	31.	.8	.8	38.	38.	33.	33.
	16.4	16.4	13.3	13.3	47.	47.	47.	47.
	10.7	10.7	22.4	22.4	10.	10.	26.	26.
	7.7	7.7	13.2	13.2	6.	6.	17.	17.
	11.6	11.6	1.3	1.3	12.	12.	4.	4.
22	5.7	5.7	7.0	7.0	7.	7.	4.	4.
	8.5	8.5	10.5	10.5	7.	7.	12	12.
	8.9	8.9	8.9	8.9	0	0	27.	25.
	0	0	4.8	4.8	0	0	6.	6.
	5.5	5.5	3.5	3.5	3.	3.	1.	1.
	.9	.9	14.4	14.4	25.	25.	18.	18.
59	15.1	15.1	17.9	17.4	61.	61.	188.	176
	10.9	10.9	16.9		91.	91.	163.	
	1.5	1.5	16.8	14.7	15.	15.	149.	145.
			12.6				140.	
			10.4	9.2			97.	97.
			8.0				97.	
	3.7	3.7	16.8	15.4	187.	187.	316	296.
	16.2	16.2	13.9		158.	158.	275.	
		10.	12.4	11.2		173.	256.	227.
			9.9				197.	
			9.1	8.7			182	147.
			8.3				112	
12 & 147	1.5	4.5	8.4	8.4	15.	15.	46.	46.
	11.3	11.3	1.5	1.5	14.	14.	73.	73.
	5.1	5.1	9.6	9.6	17.	17.	22.	22.
	9.5	9.5	16.6	16.6	33.	33.	12.	12.
	15.9	15.9	3.6	3.6	31.	31.	7.	7.
	9.9	9.9	4.2	4.2	6.	6.	12.	12.
165	10.4	10.4	37.0	36.1	21.	21.	156.	119.
	5.7	5.7	35.0		38.	38.	82.	
	26.4	26.4	34	34.	48.	48.	34.	34.
			12.	12.			34.	34.
	1.3	1.3	11.9	11.	99.	99.	196.	189.
	10.4	10.4	10.		56.	56.	181.	
	8.1	8.1	5.3	5.3	33.	33.	154.	154.
			1.8	1.8			151.	151.

justed. Where the school influence is thus equalized in each ventilation type the average illness rates are appreciably different from the uncorrected averages. In the comparison of Types A and B only those schools have been used which possessed both A and B rooms. A B room in a school not having

TABLE XX  
COMPARATIVE RATES OF RESPIRATORY ILLNESS WITH UNCORRECTED AND BALANCED AVERAGES  
(BOTH STUDIES COMBINED)

VENTILATION TYPE	ABSENCE RATE		ILLNESS IN ATTENDANCE RATE	
	UNCORRECTED	BALANCED	UNCORRECTED	BALANCED
A	10.0	9.6	57	48
B	9.0	9.1	39	45
B	9.4	9.4	38	43
C	12.0	11.1	94	73

an A room is omitted. Similarly, in the comparisons of B and C rooms, B rooms are omitted where there is lacking a C room in the same school.

Before correcting for number of rooms the absence rates for A and B were 10.0 and 9.0. After eliminating the abnormal influence of the schools with the most rooms, the rates are 9.6 and 9.1. In the first instance Type A was greater than B, largely because of the greater number of rooms at Schools 39 and 59, where the rates are higher.

The effect of eliminating the school influence is even more noticeable with the rates for illness in attendance. Without correction the rate for A was 57 and for B, 39, an appreciable difference. When the influence of each school has been equalized, the rates are 48 for A and 45 for B. In the former instance A exceeds B only because it possessed more rooms at P. S. 59, where the rates are high, and not because of any ventilation influence.

The corrected absence rates for Types B and C are 9.4 and 11.1, a difference of 1.7. Before the correction had been applied the rates were 9.4 and 12.0, a difference of 2.6. It was the greater number of rooms at P. S. 59, where the rates are high, that raised the average for Type C. When this influence is modified the difference between the two types is less.

A marked alteration is also produced in the rates for illness-in-attendance. Without the correction for number of rooms, the figure for B is 58 and for C, 94, a difference of 56. Eliminating the school influence the rates are 43 and 73, a difference of only 30. School 59 is largely to blame for the apparent wide difference in the types. With an equal number of rooms at each school we obtain a truer conception of the difference in ventilation types.

Having arrived at comparative figures which are believed to give a fairly accurate measure of the ventilation influence, we are confronted with the interpretation of these results. Is the difference of 0.5 between the absence rate in A and B significant, or is it a chance result which, if the experiment were repeated, would reverse itself? We can answer this question by determining the probable error of the averages. If the differences are statistically significant, they will represent at least three times the value of the probable error. If the differences are no greater than the probable error, then we cannot say that ventilation exercises an unmistakable effect on the health of these school children.

The formula for the probable error is

$$P. E. = 0.6715 \sqrt{\frac{\sum N}{n}}$$

The computation of the probable error is made by averaging the absence rates in each type; finding the deviation of each rate from the average; squaring each deviation; averaging these squares; determining the square root from this average; dividing this figure by the square root of the number of cases, which gives the standard deviation and finally multiplying this figure by the constant, 0.6715, which gives the probable error, or P. E.

The difference in the absence rates between Types A and B, 0.5, is less than the probable error, or approximately 0.7, and in consequence, is without significance. This means that the evidence is insufficient to prove that either

type of ventilation is superior to the other in so far as respiratory illness is concerned.

Respiratory illness of a less severe nature and sufficient to keep children out of school is likewise, judging by the fact that the probable error exceeds the difference in rates, no different in a cold, window ventilated room with a temperature around 59 degrees than in a cool, window ventilated room whose temperature is in the neighborhood of 64 degrees. This finding is in agreement with the original computations for the entire group of rooms.

The differences between Types B and C are more marked. The higher probable error for absence rates, that is for Type C, is 1.0. The difference

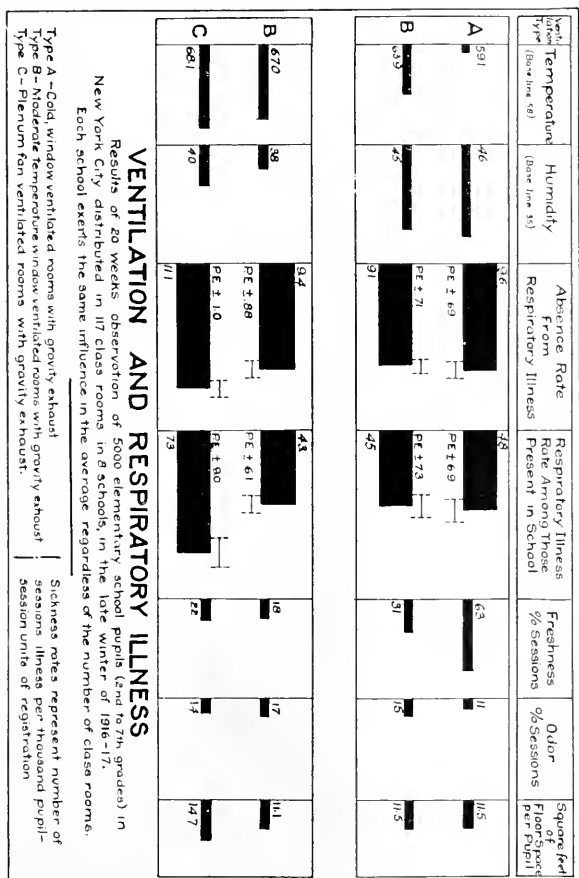


Chart III.

TABLE XXI  
PROBABLE ERROR OF RESPIRATORY SICKNESS RATES  
(BOTH STUDIES COMBINED)

	DIFFERENCE BETWEEN VENTILATION TYPES A AND B	PROBABLE ERROR	DIFFERENCE BETWEEN VENTILATION TYPES B AND C	PROBABLE ERROR
Absence Rate	0.5	A $\pm$ .69 B $\pm$ .71	1.7	A $\pm$ .88 B $\pm$ 1.00
Sickness in Attendance Rate	3.0	A $\pm$ 6.9 B $\pm$ 7.3	30	A $\pm$ 6.1 B $\pm$ 9.0

(Note: Only schools used in both studies are included in the above.)

between the sickness rates is 1.7. While this difference is not greater than three times the probable error, the mere fact that it is greater suggests at least a tendency for Type C rooms to be more conducive to respiratory illness than those of Type B.

Minor respiratory illness insufficient to cause absence amounts to a rate of 43 in Type B rooms and to 73 in Type C. This is a difference of 30. The greater probable error is 9.0. The difference is thus more than three times the probable error and statistically may be regarded as significant. Interpreted in other words the system of fan ventilation representing Type C is more conducive to respiratory illness among school children than the window ventilation methods of Type B.

In Chart III are represented graphically the absence rates as well as other facts pertinent to the matter. The difference in average temperature between Type A and B rooms was 4.8 degrees. Relative humidity was almost identical, being 46 per cent in the A rooms and 45 in B. The A rooms were judged exceptionally fresh 63 per cent of the time as against 31 per cent for B. The air possessed a noticeable odor 11 per cent of the time in A and 15 per cent in B. The degree of congestion was the same in both types, namely: 11.5 square feet of floor space per pupil.

In the second comparison, the Type B rooms averaged 67.0 degrees temperature as against 68.1 for the C rooms, a difference of but 1.1 degrees. Relative humidity was 38 per cent in B and 40 per cent in C. The B rooms were judged exceptionally fresh 18 per cent of the time, the C rooms 22 per cent. Odor was noticeable 17 per cent of the sessions in B and only 14 in C. The B rooms were appreciably more congested, the square feet of floor space per pupil being 11.1 as against 14.7 in the C rooms.

The following conclusions appear justified from the evidence:

1. Respiratory sickness is no greater in a window ventilated schoolroom kept around 59 degrees than it is in a room where temperature is 64.

2. Respiratory sickness is greater in fan ventilated rooms, such as are represented in this study, than in window ventilated rooms, even though there is not more than a degree difference in temperature, and the fan rooms are more spacious.

3. It is low temperature rather than chemical purity of the air which conveys the sensation of freshness.

## LABORATORY METHODS

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### A COLORIMETRIC METHOD FOR THE ESTIMATION OF MORPHINE IN COLLOIDAL MIXTURES AND TISSUES\*

BY HARRY GAUSS, M.D., DENVER, COLO.

THE necessity for a rapid, accurate method of analysis for morphine frequently arises in toxicological, as well as in work in experimental therapy. It was during the course of investigations on the effect of morphine upon experimental tuberculosis that a survey of the literature revealed the necessity of further study to develop such a method. It was rather noteworthy that in spite of the many color reactions for morphine that are available, which are mostly evanescent and unstable, there was found no suitable colorimetric method applicable to a rapid and accurate determination of this alkaloid in tissues.

By elimination from a large number of color reactions for morphine, two were chosen as being the most suitable for the purpose at hand, the one the reaction with Marquis' reagent (a mixture of one part of pure 40 per cent formaldehyde in 15 parts of concentrated sulphuric acid (C. P.) giving a purple-red color with morphine which gradually but slowly changes to violet and then to a fairly permanent blue; and the other the reaction with Lafon's reagent (a solution of a selenite or selenic acid 0.5 per cent in concentrated sulphuric acid (C. P.) giving an evanescent blue to intense blue with this alkaloid and finally a fairly persistent olive green. The latter reagent has recently been recommended by Morgulis and Levine<sup>1</sup> and has been used not as a quantitative colorimetric method but as an extinction point method giving only relatively quantitative results. The reagent was found by them to be sensitive to 0.025 milligram morphine sulphate with a positive reaction, while they could still recognize 0.0125 milligram of the pure alkaloid salt. When mixed with tissues no less than 0.5 milligram of morphine sulphate (0.2 mg. of the alkaloid) could be detected. Their method of extraction was to heat the tissues with 2 per cent tartaric acid, in proportion of 20 grams of substance to 100 c.c. of reagent heating for 30 minutes on the water-bath, cooling and straining through cheese cloth, uniting the collected washings after the last is free from acid, evaporating to a thick consistency, adding sodium bicarbonate until effervescence ceases and then adding a slight excess. The alkaloid thus being set free is extracted from the dried ground mass by means of chloroform using 50 c.c. for the extraction. Unfortunately the margin of error of their method is entirely too great to give more than approximate results.

\*From the Research Department, National Jewish Hospital for Consumptives, Denver, Colorado.

Heidreschka and Faul<sup>2</sup> have used the Marquis' reagent for colorimetric purposes and have found it sensitive quantitatively in concentrations varying from 1 in 500 to 1 in 25,000. The colors are examined by transmitted light, since in reflected light an actual color change from blue to bluish brown renders the comparison untrustworthy. Their determinations were made on poppies. They also used a colorimetric method described by Georges and Gascard<sup>3</sup> in which a yellow coloration is obtained by means of iodic acid added to solutions of morphine in dilute one tenth normal hydrochloric acid. Quantitative observations with a Dubosecq colorimeter using this color test can only be made at concentrations between 1 in 500 and 1 in 5000 with this reagent.

Colorimetric methods are convenient on account of the rapidity with which analyses can be made, the fair accuracy attainable and the ease of comparison with a standard of a pure sample of the substance being analyzed for. Most colorimetric methods depend upon the formation of a stable colored compound but are applicable to certain unstable colors provided they change only slowly.

During the course of investigations, necessity occurred for a fairly accurate rapid quantitative method of extraction of morphine from colloidal mixtures (such as agar) and tissues and subsequent rapid determination. The usually described methods proved cumbersome and many were totally inadequate. It was for these reasons that the following detailed investigations were carried on. Unfortunately no stable color is developed by morphine as far as could be found in the literature but the most suitable color was found to occur with Marquis' reagent and this color is evanescent. This, however, necessitated the use of a standard of known amount of morphine to which the reagent was added simultaneously to a preparation of the unknown. Since, however, the color changes occur with equal speed especially with approximately equal amounts of morphine, this proved entirely satisfactory. It also proved to be quite flexible as is shown by the following experiment.

TABLE I

THE COLOR REACTION OF MORPHINE SULPHATE WITH MARQUIS' REAGENT IN PROPORTION TO THE AMOUNT OF MORPHINE BY WEIGHT

MORPHINE SULPHATE BY WEIGHT		COLORIMETER READINGS	
Tube I	Tube II	Tube I	Tube II
1 mg.	1 mg.	10.0	10.0
1 mg.	2 mg.	10.0	5.0
1 mg.	3 mg.	10.0	3.3
1 mg.	4 mg.	10.0	2.5
1 mg.	5 mg.	10.0	2.0

Solutions containing 1, 2, 3, 4 and 5 milligrams of morphine sulphate were placed in separate beakers and evaporated to dryness on the water-bath. To each of two beakers was added simultaneously 10 cubic centimeters Marquis' reagent and the colors dissolved by stirring with a glass rod. An assistant is employed to mix the color in one of the beakers to insure a simul-



taneous reaction. It was thus found that the depth of color varied directly with the proportion of morphine sulphate as is indicated in Table I. Color comparison is inversely proportional to the amount of morphine sulphate by weight.

The Marquis' reagent used in these experiments was always prepared fresh just before use from C. P. reagents—formaldehyde solution and sulphuric acid. The reagent must be perfectly colorless. The amount of Marquis' reagent used in each test was 10 c.c. In control tests using from 1.0 milligram to 0.005 milligram of morphine sulphate, it was found that 0.05 milligram could be determined with reasonable accuracy in the colorimeter\* as is shown in Table II.

TABLE II  
THE LIMIT OF DELICACY OF THE COLORIMETRIC TEST FOR MORPHINE SULPHATE

MORPHINE SULPHATE BY WEIGHT		COLORIMETRIC READING (WITH UNIT VOLUME DILUTION—10 C.C.)	
Tube I	Tube II	Tube I	Tube II
1 mg.	0.50 mg.	10.0	20.0
1 mg.	0.25 mg.	5.0	20.0
0.2 mg.	0.10 mg.	10.0	20.0
0.1 mg.	0.05 mg.	10.0	20.0

Since the extinction point with the Marquis' reagent is far below the quantitative colorimetric range as determined in a colorimeter a study of this phase seemed desirable. For comparison the extinction point of morphine sulphate with Lafon's reagent (selenium-sulphuric acid) was also determined since at times it is desirable in determining small amounts of morphine to use two tests. The results of the study of the extinction point for Marquis' and Lafon's reagents are given in Table III.

The comparison of the extinction points of morphine sulphate with Mar-

TABLE III  
THE EXTINCTION POINT OF MORPHINE SULPHATE WITH MARQUIS' AND LAFON'S REAGENTS

AMOUNTS OF MORPHINE SULPHATE TESTED BY WEIGHT	RESULTS	
	WITH MARQUIS' REAGENT (HCOH H <sub>2</sub> SO <sub>4</sub> )	WITH LAFON'S REAGENT (H <sub>2</sub> O H <sub>2</sub> SO <sub>4</sub> )
0.025 mg.	--	--
0.010 "	--	--
0.009 "	--	--
0.008 "	--	-- definite green blue color
0.007 "	-- definite red color	--
0.006 "	±	--
0.005 "	--	--
0.004 "	-- less marked red color	-- still a slight green blue color
0.003 "	-- still visibly red	± doubtful reaction
0.002 "	± doubtful reaction	--
0.001 "	--	--

\*The marking --, ±, + and ++ indicate the intensity of the color reaction obtained with 1 c.c. of the reagent—usually in the extinction method the color appears only at the site of the morphine and not diffused throughout the reagent. These results are the average of three readings on the same amount of morphine sulphate.

\*Throughout this entire investigation the Kober Colorimeter-Nephelometer was used.

quis' and Lafon's reagent reveal very little difference in the sensitiveness of the two reagents although the Marquis' reagent is slightly more delicate.

In order to further test the colorimetric method for morphine sulphate using the color developed with the Marquis' reagent some commercial one-eighth grain hypodermic tablets of morphine sulphate obtained from two different firms were analyzed as follows: A tablet was pulverized in a beaker, to it was added 50 cubic centimeters of fresh Marquis' reagent, the mixture being well stirred to obtain a uniform color. This solution was then compared to a standard made by dissolving one milligram of chemically pure morphine sulphate in 10 cubic centimeters of Marquis' reagent. The depths of color were compared in the Kober colorimeter, with the final computed contents of the tablets (3 of each make) as given in Table IV.

TABLE IV

THE COLORIMETRIC ANALYSIS OF ONE EIGHTH GRAIN HYPODERMIC TABLETS OF MORPHINE SULPHATE BY THE USE OF MARQUIS' REAGENT

TESTS	TABLETS PREPARED BY FIRM IN	
	INDIANAPOLIS	BALTIMORE
1	7.77 milligrams*	7.56 milligrams
2	7.46 milligrams	7.46 milligrams
3	7.62 milligrams	7.93 milligrams

\*These tablets should theoretically contain 8.1 milligrams of morphine sulphate.

Thus far our analyses have been for the pure alkaloid (combined with inorganic radicals or mixed with other inorganic salts) not mixed with organic colloidal matter or tissues. When present in the latter, recourse must first be had to methods of extraction. Since the extraction from agar-agar was of interest to us in certain chemotherapy studies, extraction from this colloid was first studied. It also seemed that with slight modification a method applicable to agar could easily be used for tissues. Most methods of extraction of morphine from organic material contemplate first forming the alkaloidal salt, obtaining it in aqueous solution and finally extracting the pure alkaloid by means of an alkaloid solvent such as chloroform, after the salt has been decomposed by the addition of an appropriate alkali.

As a result of some preliminary tests it appeared that the crucial point in the quantitative recovery is the extraction with chloroform and for this reason several modifications of chloroform extraction were tested.

Four ten cubic centimeter portions of a solution, each containing 5 milligrams of morphine sulphate, were used. Two of these were alkalinized with ammonium hydroxide and two with sodium bicarbonate, one of each of these two sets was extracted with cold chloroform and the other with hot chloroform—each extraction being made with 10 cubic centimeters of chloroform. The chloroform extracts were all separated in a separatory funnel and each extraction analyzed for its morphine sulphate content. The results of the individual extractions and number necessary to completely extract the 5 milligrams of morphine sulphate are given in Table V.

TABLE V

AN ANALYSIS OF THE CHLOROFORM EXTRACTIONS NECESSARY TO RECOVER 5 MILLIGRAMS OF MORPHINE SULPHATE FROM AQUEOUS SOLUTION UNDER CERTAIN CONDITIONS

NUMBER OF EXTRACTION	ALKALIZED WITH			
	AMMONIUM HYDROXIDE		SODIUM BICARBONATE	
	EXTRACTED WITH COLD CH <sub>2</sub> Cl <sub>2</sub>	EXTRACTED WITH HOT CH <sub>2</sub> Cl <sub>2</sub>	EXTRACTED WITH COLD CH <sub>2</sub> Cl <sub>2</sub>	EXTRACTED WITH HOT CH <sub>2</sub> Cl <sub>2</sub>
1	0.115	0.220	1.666	2.500
2	0.356	0.243	1.052	1.818
3	0.143	0.266	0.833	0.294
4	0.133	0.266	0.500	0.147
5	0.433	0.215	0.454	0.098
6	0.414	0.233	0.333	0.098
7	0.227	0.225	0.100	Trace
8	0.080	0.266	Trace	Trace
9	0.123	0.416	Trace	0
10	0.100	0.209	0	4.955
11	0.066	0.225	4.938	
12	0.100	0.200		
13	0.107	0.234		
14	0.147	0.294		
15	0.147	0.225		
16	0.075	0.171		
17	0.098	0.215		
18	0.113	0.215		
19	0.098	0.107		
20	0.110	0.178		
21	0.133	0.125		
22	0.215	0.125		
23	0.215	0.075		
24	0.215	Trace		
25	0.187	Trace		
26	0.107	0		
27	0.115	4.918		
28	0.147			
29	0.125			
30	0.125			
31	0.125			
32	0.090			
33	Trace			
34	Trace			
35	0			
	4.984			

This experiment indicates that of the methods tested the extraction with hot chloroform after alkalinizing with sodium bicarbonate is the most efficient, requiring only eight extractions of 10 c.c. each. As a result of all these preliminary tests the final method used for the analysis for morphine sulphate in nutrient agar was as follows:

The nutrient agar is broken up as well as possible into small pieces and is mixed with 2 per cent tartaric acid solution in proportion of 5 times

as much reagent as agar. The entire is well mixed and allowed to stand overnight after which the soluble tartrate is filtered off, the agar residue being washed about three or four times with distilled water. The combined filtrate and washings are evaporated over a water-bath down to 10 to 15 c.c. to which is added dry sodium bicarbonate slightly in excess of the point where effervescence ceases. To this is added 10 c.c. of pure chloroform, the mixture shaken and heated for 5 minutes over an electric hot plate. For this purpose a simple modified Wiley condenser is used to prevent evaporation and loss of the chloroform. After cooling, the chloroform layer is removed in a separatory funnel and the aqueous part is extracted for 7 or 8 times more. The total chloroform extracts are combined, diluted to a definite volume, and fractions thereof used for colorimetric comparison after evaporating off the chloroform and adding Marquis' reagent to the residue. A point in technic that has been important in obtaining satisfactory results is that no trace of acid be present in the glassware into which the chloroform extracts are placed. In order to avoid this error all the glass utensils were rinsed just before use with a small amount of weak ammonia water.

### *Colorimetric Analysis and Determinations*

The colorimetric analysis and determinations were made as follows:

1. The standard of morphine sulphate is prepared from an aqueous solution containing 1 milligram per one cubic centimeter. This is placed in a small beaker and evaporated to dryness on the water-bath. A portion of the unknown in chloroform is placed in another beaker and likewise evaporated to dryness. When both residues, the known and unknown have cooled to room temperature, 10 cubic centimeters of a freshly prepared Marquis' reagent are added to each simultaneously and stirred until the color is uniformly diffused throughout the fluid. An assistant is employed to stir one of the beakers. If the color of the unknown is considerably deeper than the known, definite amounts of Marquis' reagent can be added until the colors more nearly match, or vice versa. The colors are compared in the colorimeter (in this work the Kober colorimeter proved entirely satisfactory) and the results are calculated with the following formula.

$$\frac{K}{X} \cdot S \cdot N \cdot \frac{V}{P} = \text{Mg. alkaloid in unknown as morphine sulphate.}$$

- K    the reading of the standard tube, this may conveniently be placed at 10.0;  
 X    the reading of the tube containing the unknown;  
 S    the strength of the standard solution. If 1 mg. of morphine sulphate has been dissolved in 10 c.c. Marquis' reagent, then the standard solution is 0.1 mg. alkaloidal salt per c.c. of solution;  
 N    the number of c.c. of Marquis' reagent used to dissolve the unknown;  
 V    the total chloroform extract and,  
 P    the portion of the total chloroform extract used in the test.

*Example.* The total combined chloroform extract is 100 c.c., of this 15 c.c. is evaporated to dryness and the residue dissolved in 20 c.c. Marquis' reagent. The standard solution contains 1 mg. of the alkaloid salt in 10 c.c. reagent. The

standard tube is set at 10 and the unknown reads 12.5. We have then  $V = 100$ ;  $P = 15$ ;  $N = 20$ ;  $S = 10$ ;  $K = 10$ ;  $X = 12.5$  or

$$\frac{10.0}{12.5} \times \frac{1}{10} \times \frac{20}{1} \times \frac{100}{15} = 10.666 \text{ mg. morphine sulphate in the unknown.}$$

By this method a series of nutrient agar tubes containing known amounts of morphine sulphate which was added to the agar have been analyzed with the results given in Table VI.

TABLE VI  
ANALYSIS AND COLORIMETRIC ESTIMATION WITH MARQUIS' REAGENT OF MORPHINE SULPHATE IN NUTRIENT AGAR OF KNOWN ALKALOID CONTENT

AMOUNT OF MORPHINE SULPHATE IN MILLIGRAMS ADDED TO 20 GRAMS NUTRIENT AGAR MEDIUM	AMOUNT OF MORPHINE SULPHATE IN MILLIGRAMS RECOVERED BY ANALYSIS	
	AGAR MIXTURE	
	No. I.	No. II.
32.0	27.2	31.5
16.0	15.5	15.3
8.0	7.6	7.8
4.0	3.5	3.7
2.0	1.97	1.92
1.0	0.94	0.96
0.50	0.42	0.44
0.10*	0.08	0.08

\*A small amount of organic matter present in the final extract giving a brownish color with  $H_2SO_4$  precludes the accurate determination of amounts below this.

The above described method of analysis for morphine sulphate in an organic colloidal mixture (nutrient agar medium) yields quantitative results down to amounts as small as 0.1 milligram in 20 grams of agar, below this amount the recovery is less perfect.

In order to test the accuracy of this method for the quantitative determination of morphine in tissues, beef muscle and pig liver to which had been added 5 milligrams of morphine sulphate were analyzed by the above described method which was slightly modified in that the tissues were mixed with 10 volumes of 3 per cent trichloroacetic acid and boiled for 10 minutes instead of mixing them with 2 per cent tartaric acid. Trichloroacetic acid

TABLE VII  
THE QUANTITATIVE ANALYSIS OF TISSUES FOR MORPHINE BY THE COLORIMETRIC METHOD WITH MARQUIS' REAGENT

SAMPLE ANALYZED	RECOVERY OF MORPHINE SULPHATE FROM 5 GRAMS OF FRESH TISSUES TO WHICH WAS ADDED 5 MILLIGRAMS OF THE ALKALOID SALT	
	BEEF MUSCLE	PIG LIVER
No. 1	4.76 milligrams	4.58 milligrams
No. 2	4.77 milligrams	4.76 milligrams
No. 3	4.67 milligrams	4.88 milligrams

was chosen because it was found in preliminary experiments to be just as satisfactory as 2 per cent tartaric acid in so far as the extraction of morphine was concerned, and as was originally found by Greenwald<sup>4</sup> it was a far more perfect tissue coagulant or protein precipitant. The results of these tissue analyses are given in Table VII.

An examination of Table VII reveals that morphine sulphate added to fresh tissues (beef muscle and pig liver) in 5 milligram amounts and immediately analyzed can be recovered practically quantitatively by the colorimetric method using Marquis' reagent as described above.

#### SUMMARY

A quantitative colorimetric method for the estimation of morphine sulphate in tissues and organic colloidal mixtures is described.

There is a preliminary precipitation of the proteins by means of ten volumes of 3 per cent trichloroacetic acid and subsequent extraction with hot chloroform.

The color utilized in this reaction is the purple red reaction with Marquis' reagent which is evanescent. The standard color is prepared by adding a known amount of the alkaloidal salt to a known volume of Marquis' reagent, similarly and simultaneously with the preparation of the unknown.

By means of this method we have been able to extract quantitatively morphine sulphate from tissues and colloidal solutions in amounts from 0.10 to 50.0 milligrams and to determine it colorimetrically in amounts as low as 0.003 milligrams.

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## THE HECHT-GRADWOHL TEST EMPLOYING ICE CHEST FIXATION. A PRELIMINARY REPORT

BY H. D. MCINTYRE, M.D., E. A. WORTH, M.D., AND A. P. MCINTYRE, A.B.  
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WHEN the ice chest method of complement fixation began to be in vogue it occurred to us that this method might yield good results in the Hecht-Gradwohl test. We had seen that the Wassermann reaction employing complement fixation in the ice chest at 2° C. yielded a considerable number of positives over the water-bath Hecht-Gradwohl test even though acetone-insoluble antigen were used.<sup>1</sup> We then decided on a comparison of the relative merits of the Wassermann and Hecht-Gradwohl test with the complement fixation taking place in the ice chest in both.

The following technic was worked out:

## TECHNIC OF THE HECHT-GRADWOHL TEST WITH COMPLEMENT FIXATION IN THE ICE CHEST AT 2° C.

Eighteen tubes are used in each test. Ten tubes are placed in the first row. These tubes are used to determine the hemolytic index as outlined by Gradwohl.<sup>2</sup> Four tubes are used in the second row, three containing plain antigen and the remaining tube being the control. Four tubes are used in the third row, three containing graded amounts of cholesterolized antigen and the other tube being the control.

Each of the eighteen tubes receives .1 c.c. of the patient's serum, not over twenty-four hours old. The best results are obtained with fresh serum. The first tube of the first row receives .9 c.c. of .9 per cent salt solution, the second tube .8 c.c., the third tube .7 c.c. and so on, the last tube in that row receiving no salt solution.

The second row receives plain antigen as follows: the first tube two units of antigen, the second tube one and one-half units, the third tube one unit, the fourth tube receives no antigen as it is the control. All tubes are brought to a volume of one c.c. with salt solution.

The third row receives cholesterolized antigen as follows: two units in the first tube, one and one-half units in the second tube, and one unit in the third tube. The fourth tube receives no antigen as it is the control. All tubes are then brought to a volume of one cubic centimeter with salt solution.

All tubes are now placed in the ice chest for a period of ten hours at 2° C. This allows the complement to be fixed in the antigen containing tubes in case the serum is a positive one. At the end of the ten hour period the ten tubes of the first row are removed from the ice chest and the hemolytic index of the serum is determined in the manner outlined by Gradwohl, that is, .1 c.c. of a 5 per cent suspension of washed sheep cells is added to the first tube, .2 c.c. to the second tube, .3 c.c. to the third tube and so on, the tenth tube receiving 1 c.c. of the suspension of cells. These tubes are then placed in the water-bath for thirty minutes at 37.5° C. The highest tube in the series showing complete hemolysis is taken as the index. Following the determination of the index the antigen containing tubes are removed from the ice chest and one-half of the amount of cells represented by the index is added to each tube in the second and third rows. The tubes are then placed in the water-bath for one-half hour at 37.5° C. If all tubes clear, the test is negative. If no hemolysis occurs in the antigen containing tubes, the control being clear, the test is positive.

One hundred sera were subjected to the foregoing technic together with the classical Wassermann with complement fixation in the water-bath and ice chest and the classical Hecht-Gradwohl with complement fixation in the water-bath. Fifty of these sera were from luetic patients, fifty were from nonluetic patients. The sera from the nonluetic patients reacted negatively to all tests. The comparative percentages of the positive reactions yielded by the different methods employed in the examination of the fifty luetic sera are given in Table I.

It will be seen that the Hecht-Gradwohl test employing complement fixation in the ice chest yields a higher percentage of positive reactions than do

TABLE I

	PERCENTAGE OF POSITIVE
1. The Wassermann test employing complement fixation in the water-bath for thirty minutes at 37.5° C. using plain antigen .....	23.07
1'. The Wassermann as in (1) except that cholesterolized antigen is used.....	34.20
2. The Hecht-Gradwohl test with complement fixation in the water-bath at 37.5° C. for thirty minutes using plain antigen.....	34.20
2'. The Hecht-Gradwohl test as in (2) except that cholesterolized antigen is used.....	53.08
3. The Wassermann test employing complement fixation in the ice chest at 2° C. for ten hours using plain antigen .....	73.00
3'. The Wassermann test as in (3) except that cholesterolized antigen is used.....	77.00
4. The Hecht-Gradwohl test employing complement fixation in the ice chest for a period of ten hours at 2° C. using plain antigen .....	80.70
4'. The Hecht-Gradwohl test as in (4) except that cholesterolized antigen is used.....	86.90

any of the other methods above tabulated. There are several reasons why this is so.

Complement in human serum is sometimes an unstable thermolabile substance which will deteriorate even if subjected to a temperature of 37.5° C. for a period of one-half hour. In the Hecht-Gradwohl test this is what happens if the complement fixation takes place in the water-bath. We have encountered ten sera in which such deterioration of complement has occurred. This was determined by placing a serum in the water-bath for one-half hour titrating a portion of it at the same time and titrating a second portion which had been subjected to the water-bath temperature. Ten sera yielded a lower index on the second titration than on the first. This source of error is obviated if complement fixation is carried out in the ice chest as the antigen containing tubes are then subjected to the water-bath temperature for only one-half hour.

Furthermore, this test has all of the added advantages pointed out by Gradwohl in addition to the one just alluded to, as well as the advantage of ice chest methods of complement fixation which we have emphasized in an earlier paper.<sup>1</sup> Theoretically the Hecht-Gradwohl test with ice chest complement fixation should be the test par excellence for the detection of antiluetic amoceptor in human serum. Practically, however, in a large series of cases, the ice chest Wassermann and the ice chest Hecht-Gradwohl tests would agree in nearly all instances.

A glance at our table will show that our percentage of positive reactions in the series of the fifty luetic patients is comparatively low. The reason is as follows: the fifty patients selected for the experiment represented cases of latent lues and lues under treatment where we had reason to know from previous serologic examination that their serums contained only small amounts of the antiluetic reacting substance. Such a series serves well to show the comparative value of serologic reactions, whereas if secondary untreated cases had been used, all the various methods would have yielded positive reactions.

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# THE PRECIPITIN REACTION USED AS EVIDENCE FOR THE IDENTIFICATION OF HUMAN BLOOD IN AN AMERICAN COURT

BY JOHN B. EKELEY, PH.D., SC.D., BOULDER, COLORADO

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THE writer is not aware of any recorded case in which the results of the application of the precipitin reaction have been used in an American court of justice as evidence for the identification of human blood. Many cases are recorded of its use in Europe, especially in Germany. It therefore seems proper that the following case in the recent experience of the writer should be available for reference.

In September, 1914, J. T., a rancher living near Raton, New Mexico, was charged\* with murdering his wife. The evidence against him was all circumstantial, a part of which was a "jumper" and overalls which he had worn on the day of the crime, and from which it was charged attempts had been made to remove blood spots by cleaning, and a carefully cleaned iron poker which the State charged had been used in committing the crime.

The writer was asked to determine whether or not the spots on the clothing were due to the presence of human blood, and also to examine the poker for any traces thereof.

Examination of the clothing showed thirty-four suspected spots, among which were several minute dark red, shiny globules adhering to roughened portions of the lower part of the leg of the overalls. The spots were carefully cut from the fabric, the excess of cloth cut away, and carefully shredded to pieces by means of a needle. The poker was made of a piece of iron rod one end of which had been bent and twisted around part of the remainder so as to form the handle. It had the appearance of having been carefully cleaned. Examination with a magnifying glass, however, showed in one of the depressions between the twisted metal a small amount of dark red, shiny material which had the appearance of dried blood. This was picked out carefully with a needle. It weighed .019 grams.

*Examination of the Spots.*—The finely shredded cloth containing the spots from the clothing was placed in a sterilized test tube together with 2 c.c. physiologic salt solution containing a trace of phenol. After remaining in an incubator at body temperature for 48 hours, the liquid was filtered off through a hard filter by suction, giving a water clear filtrate of neutral reaction. This was divided into two equal portions. To one portion was added 1 c.c. of rabbit's serum† immunized to human blood and sensitive to dilutions of 1:3000. The reaction was characteristic, the cloudiness forming within a few moments, with the subsequent coagulation and settling to the bottom of the tube. The immunized serum gave no precipitate with the physiologic salt solution alone, with diluted rabbit's blood, with guinea pig's

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\*The case was bitterly fought, dragging through six years, coming to trial twice, the first trial resulting in conviction, the second, granted by the Supreme Court after appeal, resulting in acquittal.

†The immune serum used was obtained by Dr. R. C. Whitman of the University of Colorado Medical School from Prof. Ludwig Hektoen of Chicago.

blood, or with dog's blood. To the other portion of the filtrate was added serum from a rabbit which had not been immunized against human blood. No precipitate appeared.

The conclusion was that the spots contained human blood and testimony was so given. Counsel for the defense took care on cross examination to bring out the fact that the precipitate might have been caused by the presence in the spots of dried body secretions other than blood, naming specifically serum from the nose, and seminal fluid. The fact that the blood of an anthropoid ape would also give the reaction was also brought out, but on the whole the defense did not attempt to throw any doubt upon the reliability of the precipitin reaction.

*Examination of the Material from the Poker.*—The material scraped from the poker was similarly extracted with 1 c.c. physiologic salt solution at body temperature and filtered to a water clear filtrate. This was also neutral in reaction. Addition of .1 c.c. of the immunized serum gave a characteristic precipitate as before, the conclusion being that the material from the poker contained human blood and testimony was so given.

## MERCURY MANOMETER FLOAT THAT WILL RIDE ON THE SURFACE OF THE MERCURY THE SAME AT ALL TIMES\*

BY M. A. BLANKENHORN, M.D., AND E. J. WARNICK, CLEVELAND, OHIO.

THIS mercury manometer float is made of hard rubber and has three very good features:

1. The conical shape, which on each upward stroke will rid itself of the mercury which happens to get above the float.



Fig. 1.

2. The flat base, which rides on the surface of the mercury.

3. The projection on the bottom, with a very small neck, which makes the mercury form a collar around it, thereby holding the float.

\*From the Medical Research Laboratory, Lakeside Hospital, Cleveland, Ohio.

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## EDITORIALS

### *Notes on the Vitamins*

PLIMMER<sup>1</sup> observed four young pigs presenting the following symptoms: (1) Arrest of growth. (2) Evidence of pain leading to frenzy when touched. (3) Marked lassitude and absence of appetite. (4) Spasmodic twitching of limb muscles. (5) Swollen joints. (6) Failure to coordinate movements and dragging of the limbs in some cases.

On inquiry it was ascertained that these animals were being fed on a mash composed of turnips, meal and grits, all thoroughly cooked. Cooking of the food was discontinued and the same mash, with the addition of a larger proportion of turnip fed in the raw state. The turnips were fed directly after being plucked from the fields. Occasionally some skimmed milk or butter-milk was added to this food. After a few meals of raw food the appetite showed improvement and after fourteen days the pig least affected seemed quite normal. The others recovered slowly, but within from six to eight weeks, all were accumulating fat and growing normally. Plimmer states that this disease is fairly common among pigs in England, that it is frequently referred to as "rheumatism," sometimes as "rickets" or "pig gout," and that it has been found that raw potatoes generally cure it. In due time these

animals were sold at current prices and were slaughtered. The bones of one came under Plimmer's observation and he found that, while the ribs on one side appeared normal, on the other, one showed a healed fracture and five showed hemorrhage; two of these were curved. The leg bones on one side were normal, while those on the other showed thickening of the radius and ulnar and fusion and thickening of the tibia and fibula.

Hart, Steenbock and Ellis<sup>2</sup> have published an investigation of the antiscorbutic potency of milk powders. There are three processes commercially operative in the preparation of milk powder: (a) The spray process as employed by Merrell and Soule; (b) the spray process in which the powder is removed and cooled a few seconds after being dried, employed by the California Central Creameries; (c) the drying of milk on heated rolls, known as the Just process. In testing the antiscorbutic potency of these milk powders, several things must be taken into consideration. In the first place, if the milk be poor on account of inadequate and improper food supplied to the cow it is evident that converting it into a powder will not increase its vitamin content. In the second place, under natural conditions the vitamin content of summer milk, especially when the cows feed upon green pastures, is greater than that of winter milk. It is reasonable, therefore, to conclude that any results obtained in experiments of this kind cannot be considered as having standard value. The authors summarize as follows: "(1) Milk powders vary in their antiscorbutic properties. Aside from the factor of the initial quantity of this vitamin in the milk as influenced by feeding, the powders vary in their potency with the process used in their manufacture, the spray process of manufacture being more destructive of the antiscorbutic vitamin than the Just process. (2) These results should in no way condemn the milk powders made by spray processes. They only point out their limitations when used as the sole source of nutrients in infant feeding. (3) Probably with all milk powders, irrespective of method of manufacture, the safest procedure in a restricted dietary, particularly in infant feeding, is to supplement them with some potent source of the antiscorbutic vitamin. A possible exception to this statement would apply to the powders made by the Just process from summer-produced milks or even from winter-produced milks where the cow's ration is made rich in antiscorbutic vitamin by the proper selection of roots and tubers."

We are inclined to be a little more cautious than the statements made under (3) as above quoted. When the child is fed upon milk powder, whatever the process employed in its manufacture or the season of the year when it is produced, the presence of sufficient of the antiscorbutic vitamin should be made certain by the administration of orange juice.

The same authors report their observations on the stability of the antiscorbutic vitamin and its behavior to various treatments. Numerous investigators have sought for methods of drying vegetables without reduction in their antiscorbutic properties. This investigation is especially concerned with cabbage. It was thought that possibly by drying cabbage in an atmosphere of carbon dioxide at as low a temperature as possible, its antiscorbutic vitamin might escape injury. The desiccation was carried out in such an

atmosphere at 65° C., but it was found that this does not prevent the destruction of the antiscorbutic substance. A second purpose was to study the influence of fermentation as in the manufacture of sauerkraut. Again, it was found that this process leads to the destruction of the antiscorbutic factor. Incidentally, it was ascertained that the antiscorbutic factor is destroyed, or at least greatly reduced, in the preparation of silage. This last finding is probably of more importance than that regarding sauerkraut. If the vitamin in silo is destroyed, this food becomes less valuable not only to the cattle eating it, but it affects and lowers the value of the milk as a food for children.

Drummond<sup>3</sup> comments upon the confusion which has arisen on account of the different terms employed in referring to the essential food factors which have no calorie value. In his classical paper published in 1912, Hopkins designated these bodies as "accessory factors of the diet." A little later, Funk, in his experimental studies on beriberi chose the word "vitamine." The criticism usually raised against Funk's word is that the termination "ine" is one employed in chemical nomenclature to denote substances basic in character. In order to avoid this objection it has been suggested that the word "vitamine" be written without the final "e" and this has been approved by the British Chemical Society. McCollum introduced the terms fat-soluble A and water-soluble B. This has added to the confusion. Drummond now proposes that we use vitamin A, B, C, etc. He thinks that this simplified scheme will be sufficient until these bodies are isolated and their true nature determined.

Drummond and Coward<sup>4</sup> state that much of the confusion and apparent disagreement found among writers on vitamins is due to failure to have a properly purified basal diet absolutely devoid of vitamins. They state that commercial caseinogen contains relatively large amounts of the fat-soluble vitamin and should never be used as a part of the basic diet until it has been carefully purified. They heat their commercial caseinogen for twenty-four hours or more in shallow dishes at a temperature of 102° C., after which they subject it to prolonged extraction with both alcohol and ether. Purified rice starch is almost entirely devoid of fat-soluble A and is suitable for the basic diet without complicating and costly preliminary extraction. For the fatty constituent these investigators employ a fully hardened and refined vegetable oil, usually cotton seed oil. This consists largely of tristearin and is entirely devoid of vitamin A. Orange juice and salt mixture, as well as yeast extract, are devoid of vitamin A. The composition of the basic ration employed by Drummond and Coward is as follows:

Purified caseinogen	18	parts
Purified rice starch	52	"
Refined vegetable oil	15	"
Yeast extract	5	"
Orange juice	5	"
Salt mixture	5	"

Rats fed upon this diet differed in behavior somewhat according to their age. For experiments on growth, rats from four to five weeks old and weighing from 50 to 70 grams should be selected. On the above given diet such animals show but little growth and soon cease to grow. Any considerable increase of body

weight in these animals is interpreted as an indication that the basic ration is not sufficiently purified. Older and larger rats are unsatisfactory in testing for vitamin A and its effects.

With the above basic diet, rats weighing from 50 to 70 grams were fed, with the addition of certain nuts, namely, almonds, butter nuts, walnuts, peanuts, Brazil and Barcelona nuts. The results showed that these nuts have only small amounts of vitamin A associated with their fats. This is confirmation of the conclusion reached by McCollum and others, that vitamin A is formed largely in the green part of living plants and is not stored to any appreciable extent in seeds or other resting tissues.

These investigators, on the same basic diet, have tested for vitamin A, both in vegetable and animal fats, and state their conclusions as follows: "(1) No hard and fast line can be drawn between the animal and the vegetable oils and fats when their value as a source of vitamin A is being considered. (2) Taken as a class the animal fats possess growth-promoting power superior to that of the vegetable oils, but we have observed that one or two members of the latter class (e. g. palm oil) may show considerable activity in that respect. (3) Unless we assume the existence of a leucoform it does not appear probable that the fat-soluble vitamin is a member of the lipochrome class of pigments. The frequent association of the growth factor with pigments of that type must therefore be regarded as accidental. (4) The nutritive value of an animal oil or fat would appear to be influenced considerably by the diet of the animal. One preliminary experiment shows that the winter feeding of cows may have the effect of lowering the food value of the milk unless considerable care is exercised in the selection of the animal's diet."

Zilva<sup>5</sup> has apparently demonstrated the following: Alcohol extracts from carrots and cabbage the fat-soluble factor A; the alcoholic extract for from ten to twelve grams of fresh carrots given daily is sufficient to promote normal growth in rats subsisting on a diet wholly wanting in the fat-soluble factor; the alcoholic extracts from carrots contain the antineuritic and to a smaller extent the antiscorbutic factors; an ethereal extract from the alcoholic fraction equivalent to twenty-five grams of fresh carrots promotes recovery and renews growth in rats declining in weight on account of a fat-soluble deficiency.

Stephenson and Clark<sup>6</sup> have made a special study of the eye involvement which appears in some rats fed upon a diet deficient in vitamin A. This eye complication was first observed by McCollum and Simmonds in 1917 and was designated xerophthalmia, but it is now most frequently described under the name keratomalacia. It is worthy of mention that a similar disease was reported by Mori in Japan in 1904 among children who were suffering from fat starvation. Mori found that it was cured by feeding on chicken livers and fish oils. He made the further observation that it occurred among populations using vegetable oils but was never seen among people using fish oils. A similar disease was reported in Copenhagen by Bloch in 1917 among children fed upon highly skimmed milk. Bloch found that these children were cured by feeding upon whole milk and cod-liver oil. Stephenson and Clark find that keratomalacia occurred in twenty-eight per cent of their rats kept on a diet

free from vitamin A and that the eye complication rapidly disappeared when the deficiency was made good. They state their findings as follows: "(1) Corneal disease is not coincident with failure of growth culminating in death; it forms only twenty-eight per cent of the cases examined. (2) This disease is further differentiated from the cessation of growth-death symptoms by occurring at a later stage in the experimental period and rising more rapidly to a maximum. (3) At no period in the experiment are all the deaths preceded by eye disease or all the survivors afflicted with it. (4) Cure of the corneal disease was affected by the replacement of the fat-soluble factor in one hundred per cent of the cases attempted." They failed to find any specific bacterium associated with the local disease in the eye. Histologically, the first evidence of the eye being affected is the presence of numerous leucocytes in the cornea. This leucocytic infiltration is accompanied by or soon followed by the presence of bacteria which are diverse in kind. It appears, therefore, that the absence of vitamin A lowers the vitality of the corneal tissue and while this condition is in operation any bacteria that may be present begin operation. The cure of the eye complication is in no way hastened or modified by local application, but disappears wholly within a few days after return to normal diet. It will be understood that the keratomalacia may go so far as to lead to perforation of the cornea and destruction of the eyesight. Of course, return to proper food after this stage has been reached will not restore the sight, but does arrest the inflammatory processes. This appears to be a striking instance of the relation between lowered nutrition and bacterial infection. In this case the nutrition of a specific tissue is so reduced by lack of an essential food factor that any bacterium which finds access to the tissue in this condition takes on pathogenic qualities.

Stephenson<sup>7</sup> has studied the relation between certain yellow plant pigments and vitamin A. In 1914 it was shown by Palmer and Eckles that the coloring matter in milk and in the body fats of cows consists essentially of carotin with only traces of xanthophyll. The same substance is found to be the chief coloring matter in the fats of certain other herbivorous animals, especially the horse. Strange to say, however, carotin cannot be found in the blood serum or fats of swine and only traces in the corresponding tissues of sheep and goats. The pigments stored by birds and found in the yolk of the egg and in the body fats is principally xanthophyll. Quite naturally, one comes to regard a yellow milk as a rich milk and is inclined to judge of the food value of the milk by its color. The researches of Stephenson seem to show quite conclusively that vitamin A is not carotin and that the color of milk is not an indication of its richness in vitamin A; in fact, the coloring matter of butter fat may be completely removed or destroyed by filtration through charcoal without in the least affecting the vitamin content of the butter.

Hopkins<sup>8</sup> has shown that vitamin A in butter is highly resistant to heat, provided aeration is excluded. Butter may be heated to 120° C. in the absence of air for four hours and even longer without any marked reduction in its vitamin content, as demonstrated by rat feeding provided the fat forms fifteen per cent of the food; on the other hand, aeration for four hours at 120° destroys the greater part of the vitamin in butter. It will be understood that

aeration at high temperatures leads to more speedy destruction of the vitamin than aeration at low temperature, but even at ordinary room temperature butter exposed in thin layers loses much of its vitamin A within a few days. The housewife has learned by experience that butter keeps better in large masses at low temperatures and with the exclusion of light. It seems that we have unconsciously through generations learned many things as to the preparation and preservation of food which only recently science has demonstrated.

Since vitamin A is destroyed by aeration, it is plain that exposure of this food to an ozonized atmosphere is detrimental. This has been scientifically demonstrated by Zilva, who has shown that when butter is spread on a thin layer and exposed to ultraviolet rays the fat-soluble factor is inactivated.

There has been some discussion among experts as to the presence of vitamin A in lard. Numerous experiments have been made and generally the conclusion has been reached that the body fat of the hog is devoid of this essential food factor. Drummmond and his assistants have gone through this subject again and they have found that the amount of vitamin A in lard depends to a considerable extent at least, upon the food upon which the hog has been reared and the process employed in rendering the lard. They conclude their work with the following statements: (1) "The pig is able to store up supplies of vitamin A in the body fat when fed upon a diet containing ample supplies of that factor, as for example when grass is fed. (2) When the diet of the pig is deficient in vitamin A, as for example when the diet consists almost entirely of toppings and whey, no appreciable amounts of that dietary factor can be detected in the body fat. (3) The processes employed in the manufacture of lard on a large scale cause a very marked destruction of the vitamin present in pig fat."

There have been many attempts to connect deficiency diseases with disorders of some endocrine gland. Nothing very substantial has come from these suggestions. Now, Cramer comes along with the suggestion that certain fat formations in the body are really glandular tissue and that these are concerned in some way with the functioning of the vitamins. He says: "In all types of mammals which have been investigated there exists a glandular type of adipose tissue histogenetically distinct from the ordinary adipose. It has been described under various names, e. g., 'primitive fat organ,' 'fat gland,' hibernating gland,' 'brown fat,' 'interseapular gland.' In all species it has in the embryo a characteristic gland-like structure. In some species (white rat, tame mouse, hibernating animals) it retains this characteristic histological structure. But in most species the tissue acquires the appearance of ordinary adipose tissue soon after birth, so that the process has been looked upon as one of the stages leading to the formation of the adipose tissue of the adult organism. It is shown that this tissue, which is very vascular, is functionally distinct from ordinary adipose tissue. The fatty material which it contains is rich in cholesterolin compounds and other lipoids in addition to ordinary true fat. This load of lipoid is retained under conditions which bring about the disappearance of the ordinary adipose tissue. Evidence is given to show that it is functionally related to the thyroid and adrenal glands. A particularly close relationship exists between the lipoids of this tissue and those of the adrenal



cortex. The significance of the changes of the lipoids in the adrenal cortex is discussed. If vitamins are completely withheld from the diet the lipoids disappear from the adrenal cortex and from this tissue, which persists, however, as a tissue and exhibits the appearance of a very vascular endocrine organ. The existence of this tissue throws fresh light on the problem of deficiency diseases, since disturbance of the functional activity of this gland-like tissue will have to be considered as a factor in the etiology of these diseases. In view of its appearance its function and its importance it is proposed to call this glandular type of adipose tissue the 'lipoid gland' or 'cholesterin gland.' "

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- <sup>4</sup>Biochem. Jour., 1920, xiv, 161.
- <sup>5</sup>Biochem. Jour., 1920, xiv, 494.
- <sup>6</sup>Biochem. Jour., 1920, xiv, 502.
- <sup>7</sup>Biochem. Jour., 1920, xiv, 715.
- <sup>8</sup>Biochem. Jour., 1920, xiv, 725.
- <sup>9</sup>British Jour. Pathol., 1920, i, 184.

—V. C. V.

### *Superinfection in Syphilis in Its Relation to Subtreatment*

IT is generally held that a person infected with the virus of syphilis becomes practically immune to a second infection; that, with the development of the initial lesion, a condition becomes established which makes it difficult or impossible to superimpose a second infection upon the one already present and that this refractory state is maintained as long as an infection exists. Both human and animal experiences agree upon this, so far as they have been tested. In the absence of any evidence to the contrary it has been assumed that the principles contained in this conception of syphilitic immunity apply to treated as well as to untreated cases of syphilis and many syphilographers have regarded the appearance of fresh lesions of the chancre type, under circumstances which would indicate a new infection, as the most conclusive evidence of the cure of the previous infection. But Jacobi suggested that the reaction to a second infection may be viewed more as the expression of any existing state of immunity than as evidence of the presence or absence of infection.

There are matters that need elucidation and the need is accentuated by the fact, so stated by Brown and Pearce, that until the introduction of modern methods of spirocheticidal therapy, instances of so-called reinfection were comparatively rare, and interest in the subject was largely a theoretic affair. The point at issue, say Brown and Pearce, is not so much a question of the immunity conferred by syphilitic infection as it is the effect which certain therapeutics may have upon the resistance of infected individuals, and upon any spirochetes which may survive their action. This is the problem which Brown and Pearce have approached experimentally, for the purpose of dis-

covering the effect of subcurative doses of arsphenamine and neo-arsphenamine upon the resistance of infected animals to reinoculation.

These experiments consisted of the infection and treatment of two sets of rabbits—one with arsphenamine; the other with neoarsphenamine—after which they were reinoculated with luetic virus for the purpose of determining their susceptibility to a new infection. The experiments were rigorously controlled. Animals after being inoculated with the syphilitic virus and the lesions had developed were given subcurative injections of the two drugs, after which they were subjected to reinfection.

The effect of the treatment used in the experiments was as follows: Following the administration of the drugs the lesions began to regress, resolution proceeding somewhat more rapidly in the animals treated with neoarsphenamine than in those treated with arsphenamine. The first evidences of relapse were noted between 14 and 17 days after treatment. Clinical relapse among animals treated with neoarsphenamine was more delayed than among those treated with arsphenamine. In all the animals of both series, however, relapse occurred, which proves that the infection was active, and that the treatment used subcurative.

For the effects of reinoculation of infected (but untreated) controls, animals with the most advanced lesions were selected, while those showing least progress were used as controls. In but two instances out of ten inoculations did any lesion result which gave evidence of being due to syphilitic infection.

Reinoculation of treated animals, on the other hand gave different results, i. e., all but two developed perfectly typical chancres associated with lymphadenitis. It is of interest to note that the relapses of the original lesions occurred in eight of the ten reinoculations at about the time when the superinfection lesions became well established.

The results of the treatment and reinoculation as a whole are stated as follows: Of the animals treated with arsphenamine and then reinoculated, the original lesions were completely resolved in but one instance, and relapses occurred within 33 days in four of the five animals. By reinoculation characteristic chancres were produced in all the animals of the group. The results with neoarsphenamine were not so uniform. In two animals the lesions were quickly resolved. Clinical relapses occurred in all but one animal in the group at from 14 to 24 days after treatment. Characteristic chancres were produced by a second inoculation in three of the five animals.

The results of the whole experiment stated in summary are: (1) that the treatment employed was insufficient to cure any of the therapeutic controls; (2) that the infected (and untreated) controls were highly refractory to a second inoculation; (3) that the treated animals were highly susceptible to a second inoculation and that though not cured of their original infection, reacted to the second inoculation with the formation of lesions indistinguishable from primary sores; and (4) that in certain instances the treatment given had rendered infected animals more susceptible to infection than the normal controls.

One wonders after reading these results, what effect on reinoculability mercury has, whether given alone or in conjunction with arsenic. This is suggested by the statement that evidences of reinfection were less frequent before the advent of arsphenamine therapy. One also wonders what influence upon the present generation of syphilitics the too-general undertreatment is having; and whether in the campaign for education of the laity some emphasis should not be placed on the fact that undertreatment of syphilis is perhaps as serious an affair as none, and that a few doses of arsphenamine gives little but a false sense of security.

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—P. G. W.

### *Goiter in Northern Michigan*

IT HAS long been known that goiter is unduly prevalent in certain areas roundabout the Great Lakes, both in the United States and in Canada. Marine has told us that at one time the sheep-raising industry in Michigan was threatened on account of the prevalence of this disease among these animals but that among sheep, goiter was exterminated by the substitution of native for imported salt, the former containing enough iodine to prevent the development of goiter in the animals. Before we entered the war, Marine and Kimball made a survey of goiter at Akron, Ohio, and induced the authorities to provide for the administration of an iodine to all school children. We suppose that this experiment was interrupted by the war; at least, we have had no information concerning the result.

Levin<sup>1</sup> has reported upon the examination of 1,783 unselected persons in the Townships of Torch Lake and Schoolcraft in Houghton County, Michigan. One part of this community is supplied with spring water, a second part with Lake Superior water, and a third, with well water. However, there is no evidence that these waters differ materially either in organic or inorganic content. Levin states that in all these waters the relation of calcium to sodium elements is always more than 1:1, and that this relationship occurs but rarely in any other water-supply in the United States. Levin's observations throw no light upon the influence of water in the causation of goiter, because, while there may be quantitative, there are apparently no qualitative, differences in the waters used. The people are Americans, the larger percentage of French-Canadian extraction, and they are above the average in intelligence and in living conditions. There are no marked unhygienic surroundings. Each home has plenty of sunlight, good ventilation, and a lot of fair size for gardening. In the 1,783 persons, ranging in age from newborn to sixty-one years, 1,146 had enlarged thyroids, with 682 simple goiters, 420 adenomas, and 44 colloid. Among the people examined there are only two cretins, and the mother of each

<sup>1</sup>One Thousand One Hundred Forty-Six Goiters in One Thousand Seven Hundred Eighty Three Persons: Arch. Int. Med., 1921, xxvii, 421.

of these has a goiter. There are a few families with several morons, although Levin apparently has not made any scientific mental tests. Out of the total number examined, mothers alone had goiters in 802 instances; fathers alone in 12, and both parents in 183. In regard to the possibility of goiter being due to infection, the author makes the following statement: "In analyzing those who have focal infection and develop enlargements of the thyroid, whether becoming toxic or not, I am not free to believe that infection alone, or the infective agent, causes goiter. The presence of infected teeth or infected hypertrophied tonsils, or contagious diseases, or other infections will cause in thyroid patients, thyroiditis, enlargements of adenomas, or toxin symptoms, occasionally. The infective agent very rarely does this directly, but the focal toxemia causes a secondary effect on the thyroid. The weight of evidence and observations leads me to conclude that except for a direct thyroiditis, which is not uncommon, thyroid enlargements or thyroid zones are not caused necessarily by direct infective agents. This is said with due respect to the modern writers on the subject who lean toward some infective agent in itself being responsible for thyroid zones. The future will settle the question whether direct infection, or intestinal toxemia, or just the lack of some element in the soil or water, or uncleanness in the handling of vegetables grown here is, or contains, the causative agent. The large numbers in our section who are practically normal and healthy otherwise, those who lose their enlargements of the thyroid during absence from this section, and the presence of enlargements which become more marked here than elsewhere during the periods of life when the greatest metabolic changes occur—as puberty, menopause, menstruation and pregnancy—are points in circumstantial evidence that offset much in the infective theory." We suggest to Levin that it would be a good idea to try in his community the administration of small doses of sodium iodid, as was proposed by Marine for the school children of Akron.

—V. C. V.

### *Editor Goes to National Research Council.*

Attention is called to the change in address of the Editor-in-chief from Ann Arbor, Michigan, to National Research Council, 1701 Massachusetts Avenue, Washington, D. C. All communications concerning the reading matter of the journal should be sent to Dr. V. C. Vaughan at the above-given address after September 10, 1921.

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<sup>1</sup>Billings, J. S.: Our Medical Literature, Trans. VII Intern. Med. Congress, Lond., 1881, p. 547.

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